PROCEEDINGS

OF THE

NATIONAL ACADEMY OF SCIENCES INDIA

1946

PART 1] SECTION A [VOL. 15

CONTENTS

PAGE

Constitution of Butrin, Part II. A Note
By Jagraj Behari Lal 1

Some Self-reciprocal Functions
By H. C. Gupta, Ph.D. 3

Condensation of Benzaldehyde with Resacetophenone in the presence of Caustic Alkalies
By D. S. Mittal 8

Condensation of 3: 5-Dinitro-2-Chlorobenzaldehyde with Malonic Acid in the presence of Organic Bases
By D. S. Mittal 10

Identification of Air Masses in the Tropics and Surface Temperatures
By S. L. Malurkar 12

New Aspects of Nitrogen Fixation in Solids and Origin of Soil Nitrogen
By N. R. Dhar 15

ALLAHABAD

PUBLISHED BY THE COUNCIL

Price Rs. 5 (India): Rs. 5/8 (Foreign)

INSTRUCTIONS TO CONTRIBUTORS

Articles should be as *brief* as possible. The viewpoint should be comprehensive in giving the relation of the paper to previous publications of the author or of others and in exhibiting, when practicable, the significance of the work for other branches of science. Elaborate technical details of the work and long tables of data should be avoided, but authors should be precise in making clear the new results and should give some record of the methods and data upon which they are based.

Manuscripts should be prepared with a current number of the PROCEEDINGS as a model in matters of form, and should be type-written in duplicate with double spacing, the author retaining one copy. Illustrations should be confined to text-figures of simple character, though more elaborate illustrations may be allowed in special instances to authors willing to pay for their preparation and insertion. Authors are requested to send in all drawings or other illustrations in a state suitable for direct photographic reproduction. They should be drawn on a large scale in Indian Ink on Bristol board, with temporary lettering in pencil. Great care should be exercised in selecting only those that are essential. If unsatisfactory drawings are submitted, authors may be required to have them redrawn by a professional artist. Particular attention should be given to arranging tabular matter in a simple and concise manner.

References to literature will be placed at the end of the article and short footnotes should be avoided. It is suggested that references to periodicals be furnished in some details and in general in accordance with the standard adopted for the Subject Catalogue of the International Catalogue of Scientific Literature, *viz.*, name of author, arranged alphabetically, with initials following (ordinarily omitting title of paper), year, abbreviated name of Journal, volume, inclusive pages.

Papers by members of the Academy may be sent to the General Secretary, National Academy of Sciences, Allahabad. Papers by non-members of the Academy should be submitted through a member.

No Papers exceeding seven pages of printed matter will be published in the PROCEEDINGS except with a special permission of the Council.

Every paper must be accompanied by *three copies* of a brief summary not exceeding 300 words in length.

A Proof will ordinarily be sent and the author is requested to return it with the manuscript, at his earliest convenience. All proof corrections involve heavy expenses which would be negligible if the papers are carefully revised by the authors before submission.

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
INDIA
(SECTION A)

Part 1] 1946 [Volume 15

Constitution of Butrin, Part II. A Note

By Jagraj Behari Lal

General Research Section, H. B. Technological Institute Cawnpore.

(*Received in the revised form September* 5, 1944).

In a previous communication[1] it was recorded that on methylation with excess of methyl iodide and potassium carbonate, butrin yields a substance crystallising from boiling water as tiny white needles, m.p. 224°C., and considered to be a O-dimethyl butrin, $C_{27}H_{30}O_{13}(OCH_3)_2$, $7\frac{1}{2}H_2O$ which on heating for several hours even at 140°C was considered to retain rather tenaciously half a molecule of water of crystallization. It is now shown to be O-monomethyl butrin, $C_{27}H_{31}O_{14}(OCH_3)$, $7H_2O$ which on heating either at 140° or at 110°C., in high vacuum over phosphorus pentoxide loses all the seven molecules of water of crystallization and then melts at 230-231°C. The original C and H values agree with the new idea. The abnormally high results previously obtained in Zeisel's experiment were due to the mistaken use of a sample of hydriodic acid of sp. gr. 1.90. The revised results are given below :

(Found in air dried sample : C, 45.8, 45.9; H, 6.8, 6.8; loss at 140°, 17.9. $C_{28}H_{34}O_{15}$, $7H_2O$ requires C, 45.7 ; H, 6.5 ; loss 17.1 %. In sample dried at 140° : C, 54.8, 54.8 : H, 5.9, 5.8 ; OMe, 5.1. In sample dried at 140°C under high vacuum over P_2O_5 (micro) ; C, 54.7 : H, 5.7 ; OMe, 4.9 ; $C_{28}H_{34}O_{15}$ requires C, 55.1 ; H, 5.6 : OMe, 5.1).

Similarly, ethylation of butrin with excess of ethyl iodide and potassium carbonate gives O-ethyl butrin $C_{27}H_{31}O_{14}(O C_2H_5)$, $7H_2O$ and the isometic chalkone derivative of the composition $C_{27}H_{31}O_{14}(OC_2H_5)$, $3H_2O$.

(Found in sample dried at 120° : C, 56.0, 56.1 55.9 ; H, 6.1, 6.1 ; 6.0 ; OEt, 7.0; $C_{27}H_{31}O_{14}(OEt)$ requires C, 55.8 ; H, 5.8 ; OEt, 7.2. Found in air dried sample : loss at 120°, 16.4, 16.6 ; $C_{27}H_{31}O_{14}(OEt)$, $7H_2O$ requires $7H_2O$, 16.8 %). The chalkone crystallising from dilute alcohol in bright yellow crystals., m.p. 183°, is isomeric with O-monoethyl butrin (Found in samples dried at 140° ; C.55.2, 55.4 ; H, 6.0, 6.1 ; $C_{27}H_{31}O_{14}(OC_2H_5)$ requires C, 55.8 ; H, 5.8 ; OEt, 7.2. Found in air-dried sample : loss at 140°, 8.3 ; $C_{29}H_{36}O_{15}$, $3H_2O$ requires 8.0 %).

Rao and Seshadri[2], in establishing the constitution of butrin, methylated butrin, with diazomethane obtained a monomethyl ether which they proved to be the 4'-O-methylbutrin. The data given above show that the methylation procedure using methyl iodide and potassium carbonate also yields the same compound.

The author wishes to convey his heartiest thanks to Dr. S. Dutt, D.Sc. of the University of Allahabad for his kind interest in the work.

REFERENCES

1. Lal, J. B. : J. *Chem. Soc.* 1937, 1562.
2. Rao, and Seshadri : *Proc. Ind. Acad. Sci.*, 1941, *14*, 29-43.

Some Self-reciprocal Functions

By H. C. Gupta, Ph.D.

Christ Church College, Cawnpore

(*Communicated by Dr. Gorakh Prasad, D.Sc.—Received December* 31, 1945).

1. It is known* that if the function $g(x)$ is R_ν, *i.e.* self-reciprocal in the Hankel transform of order ν, then the function defined by the equation

$$f(s) = \int_0^\infty x^{s-1} g(x)\, dx \tag{1}$$

satisfies the functional equation

$$f(s) = 2^{s-\frac{1}{2}} \frac{\Gamma(\frac{1}{2}\nu + \frac{1}{2}s + \frac{1}{4})}{\Gamma(\frac{1}{2}\nu - \frac{1}{2}s + \frac{3}{4})} f(1-s). \tag{2}$$

In fact, it is the converse of the above that has been employed by several workers† in the investigation of self-reciprocal functions by what might be called 'The Method of Functional Equation'. In this paper is given a proof of the converse, followed by the investigation by means of it of some new self-reciprocal functions.

2. Lemma. If both the integrals

$$\int_0^\infty x^{s-1} g(x)\, dx \text{ and } \int_0^\infty x^{-s} g(x)\, dx$$

converge, then indicially (*i.e.*, by some power of x)

$$g(x) = o(x^{|R(s-\frac{1}{2})| - \frac{1}{2}}) \text{ as } x \to 0 \text{ and } g(x) = o(x^{-|R(s-\frac{1}{2})| - \frac{1}{2}}) \text{ as } x \to \infty.$$

For suppose that $g(x) = O(x^\lambda)$ as $x \to 0$, where λ is real. Then by the Comparison Test the two integrals would converge at the lower limit if

$$R(\lambda + s) > 0 \text{ and } R(\lambda - s) + 1 > 0,$$

i.e., if $$\lambda + \tfrac{1}{2} \pm R(s - \tfrac{1}{2}) > 0 \text{ or } \lambda > |R(s-\tfrac{1}{2})| - \tfrac{1}{2}.$$

Again suppose that $g(x) = O(x^\mu)$ as $x \to \infty$. Then the two integrals would converge at the upper limit if $R(\mu + s) < 0$ and $R(\mu - s + 1) < 0$,

i.e., if $$\mu + \tfrac{1}{2} \pm R(s - \tfrac{1}{2}) < 0 \text{ or } \mu < -|R(s-\tfrac{1}{2})| - \tfrac{1}{2}.$$

---

* E. C. Titchmarsh : Theory of Fourier Integrals (1937), 245-54.

† R. S. Varma : Proc. Lond. Math. Soc. (2) 42 (1937)9-17.
S. C. Dhar : Jour. Ind. Math. Soc. (1940) 91.-96.

3. THEOREM. *If the function $f(s)$ defined by* (1) [*wherein the function $g(x)$ does not contain the parameter s*], *satisfies the functional equation* (2), *then the function $g(x)$ is Rv for $R(v)>-\frac{1}{2}$.*

Proof: Since the integrals defining $f(s)$ and $f(1-s)$ re convergent, hence by the lemma as $x\to 0$, $g(x)=o(x^{|R(s-\frac{1}{2})|-\frac{1}{2}})$ and as $x\to\infty$, $g(x)=o(x^{-|R(s-\frac{1}{2})|-\frac{1}{2}})$.

Now by using a known integral * for the fraction involving Gamma-function. (2) may be put

$$(s)=\int_0^\infty \sqrt{y}\, g(y)\left\{ -s-\tfrac{1}{2}\, 2^{s-\frac{1}{2}}\,\frac{\Gamma(\frac{1}{2}v+\frac{1}{2}s+\frac{1}{4})}{\Gamma(\frac{1}{2}v-\frac{1}{2}s+\frac{3}{4})}\right\} dy$$

$$(2a)\qquad =\int_0^\infty \sqrt{y}\, g(y)\, dy \int_0^\infty x^{s-\frac{1}{2}} J_v(xy)\, dx,$$

$$R(v+s)>-\tfrac{1}{2},\ R(s)<1.$$

Since as $x\to 0$, $|J_v(x)|=O(x^{R_v})$ and as $x\to\infty$, $|J_v(x)|=O(x^{-\frac{1}{2}})$,

(*i*) the x-integral in (2*a*) is absolutely convergent if $R(v+s)>-\frac{1}{2}$, $R(s)<0$ and

(*ii*) the y-integral is so when $R(v)+|R(s-\frac{1}{2})|>-1$ and $|R(s-\frac{1}{2})|>\frac{1}{2}$;

and the repeated integral is by implication existent. Hence the changed order in (2*a*) is permissible by de la Vallee Poussin's theorem † for the range $-R(v+\frac{1}{2})<R(s)<0$ in which all the four conditions (*i*) and (*ii*) are satisfied and we find that

$$\int_0^\infty x^{s-1} g(x)\, dx \equiv f(s) = \int_0^\infty x^{s-1} dx \int_0^\infty \sqrt{xy}\, J_v(xy)\, g(y)\, dy$$

$$(3)\quad \text{or}\qquad \int_0^\infty x^{s-1}\left\{ g(x)-\int_0^\infty \sqrt{xy}\, J_v(xy)\, g(y)\, dy\right\} dx = 0.$$

Since (3) holds for general values of the parameter s which occurs in the integrand only as an exponent of x (with respect to which integration is performed), it follows by an obvious modification of Lerch's theorem ‡ that the integrand is identically zero, that is

$$g(x) = \int_0^\infty J_v(xy)\, \sqrt{xy}\, g(y)\, dy,$$

whence the proposition.

---

* G. N. Watson: Theory of Bessel Functions (Camb., 1922) 391.

† T'A Bromwich Theory of Infinite Series (1925), 503.

‡ Lerch: Acta Math. 27 (1903) 339-51.

4. INVESTIGATION OF NEW SELF-RECIPROCAL FUNCTIONS.

Result 1. We first evaluate an infinite integral, viz.,

$$I \equiv 2^{1+\mu-\frac{1}{2}\lambda}\Gamma(\tfrac{1}{2}\mu+\tfrac{1}{2})\sqrt{\pi}\int_0^\infty x^{s-1}x^{\lambda-\mu}\left\{H_{\frac{1}{2}\mu}(\tfrac{1}{2}x^2)-Y_{\frac{1}{2}\mu}(\tfrac{1}{2}x^2)\right\}dx,$$

$$R(s+\lambda)<2,\ R(s+\lambda-\mu)>|R(\mu)|.$$

Proof. Using the integral representation*

$$H_{\frac{1}{2}\mu}(\tfrac{1}{2}x^2)-Y_{\frac{1}{2}\mu}(\tfrac{1}{2}x^2)=\frac{2(\frac{1}{2}x)^\mu}{\Gamma(\frac{1}{2}\mu+\frac{1}{2})\sqrt{\pi}}\int_0^\infty e^{-\frac{1}{2}ux^2}(1+u^2)^{\frac{1}{2}\mu-\frac{1}{2}}du,$$

it is easily found that

$$I=2^{2-\frac{1}{2}\lambda}\int_0^\infty x^{s+\lambda-1}dx\int_0^\infty (1+u^2)^{\frac{1}{2}\mu-\frac{1}{2}}e^{-\frac{1}{2}ux^2}du$$

$$=2^{2-\frac{1}{2}\lambda}\int_0^\infty (1+u^2)^{\frac{1}{2}\mu-\frac{1}{2}}du\int_0^\infty e^{-tu}(2t)^{\frac{1}{2}(s+\lambda)-1}dt,$$

on putting $\frac{1}{2}x^2=t$ and inverting the order of integration, which is justified on account of the absolute convergence of both the u- and t- integrals. Carrying out the integration with respect to t we have

$$I=2^{\frac{1}{2}s+1}\Gamma(\tfrac{1}{2}s+\tfrac{1}{2}\lambda)\int_0^\infty u^{-\frac{1}{2}s-\frac{1}{2}\lambda}(1+u^2)^{\frac{1}{2}\mu-\frac{1}{2}}du$$

$$=2^{\frac{1}{2}s}\Gamma(\tfrac{1}{2}s+\tfrac{1}{2}\lambda)\Gamma(\tfrac{1}{2}-\tfrac{1}{4}s-\tfrac{1}{4}\lambda)\Gamma(\tfrac{1}{4}s+\tfrac{1}{4}\lambda-\tfrac{1}{2}\mu)/\Gamma(\tfrac{1}{2}-\tfrac{1}{2}\mu)=f(s),\ \text{say},$$

where we set $u^2=v/(1-v)$ to evaluate the u- integral.

If we now use the duplication formula in Gamma-functions, it is seen that the functional equation† is satisfied if

$$\Gamma(\tfrac{1}{2}s+\tfrac{1}{2}\lambda)\,\Gamma(\tfrac{1}{4}\lambda+\tfrac{1}{4}s-\tfrac{1}{2}\mu)\equiv\Gamma(\tfrac{1}{2}v+\tfrac{1}{2}s+\tfrac{1}{4})\,\Gamma(\tfrac{1}{4}+\tfrac{1}{4}s-\tfrac{1}{4}\lambda),$$

to secure which the parameters on the two sides of the equality

$$\lambda,\ \lambda+2,\ \lambda-2\mu=v+\tfrac{1}{2},\ v+\tfrac{5}{2},\ 1-\lambda, \tag{4}$$

should be equal each to each but in any order whatsoever.

---

* G. N. Watson: loc. cit. § 10.4, eqn. (3).

† If we express this equation as $\phi(s)=\psi(s)$ the functional equation is, in fact,

$$\phi(s)\,\psi(1-s)=\phi(1-s)\,\psi(s).$$

The equation (4) admits of five solutions, one of which is $\lambda=\nu+\frac{1}{2}$, $\mu=\nu$, yielding the R_ν function

$$\phi(x)\equiv \sqrt{x}\{H_{\frac{1}{2}\nu}(\tfrac{1}{2}x^2)-Y_{\frac{1}{2}\nu}(\tfrac{1}{2}x^2)\}.$$

Since the function $\phi(x)$ is $O(x^{\frac{1}{2}\pm\nu})$ as $x\to 0$ and $O(x^{\nu-\frac{3}{2}})$ as $x\to\infty$, the application of the theorem is valid when $-\frac{1}{2}<R(\nu)<\frac{1}{2}$. But since either side of the integrale quation

$$\phi(x)=\int_0^\infty \sqrt{xy}\, J_\nu(xy)\,\phi(y)\,dy,$$

is an analytic function of ν if $\frac{3}{2}>R(\nu)>-1$ the function

$$\sqrt{x}\{H_{\frac{1}{2}\nu}(\tfrac{1}{2}x^2)-Y_{\frac{1}{2}\nu}(\tfrac{1}{2}x^2)\} \tag{5}$$

is R_ν for the range $\frac{3}{2}>R(\nu)>-1$.

Of the four other solutions, namely, the sets of values $(\frac{1}{2}, 0, 0)$, $(\frac{1}{2}, -2, 2)$ $(-\frac{1}{2}, -1, -1)$ and $(-\frac{1}{2}, 1, -3)$ of the parameters (λ, μ, ν) the first provides only a special case of (5), while the rest give values to ν beyond the range of validity.

Result 2. If $a=b$ and $\mu=\nu$ the ${}_2F_1$ in the value of the Weber-Schafheitlin integral* can be summed up by means of the formula

$${}_2F_1(\alpha, \beta; \alpha-\beta+1; -1)=\sqrt{\pi}\,\Gamma(\alpha-\beta+1)/2^\alpha\,\Gamma(\tfrac{1}{2}\alpha+\tfrac{1}{2})\,\Gamma(\tfrac{1}{2}\alpha-\beta+1);$$

when we are led by a slight change in the parameters to the integral

$$\int_0^\infty y^{s+\lambda-1} J_\rho\left(\frac{y^2}{2\sqrt{2}}\right) K_\rho\left(\frac{y^2}{2\sqrt{2}}\right) dy = \frac{\Gamma(\frac{1}{2}\rho+\frac{1}{8}s+\frac{1}{8}\lambda)\,\Gamma(\frac{1}{4}s+\frac{1}{4}\lambda)}{2^{\frac{1}{4}-\frac{5}{4}s-\frac{5}{4}\lambda}\Gamma(\frac{1}{2}\rho+1-\frac{1}{8}s-\frac{1}{8}\lambda)},$$

$$R(s+\lambda+2\rho)>|R(2\rho)|.$$

It follows by the theorem that the function $y^\lambda J_\rho(y^2/2\sqrt{2})\ K_\rho(y^2/2\sqrt{2})$ is R_ν if the functional equation

$$\Gamma(\tfrac{1}{8}s+\tfrac{1}{8}\lambda)\,\Gamma(\tfrac{1}{8}s+\tfrac{1}{8}\lambda+\tfrac{1}{2})\,\Gamma(\tfrac{1}{2}\rho+\tfrac{1}{8}s+\tfrac{1}{8}\lambda)\,\Gamma(\tfrac{1}{2}\rho+\tfrac{7}{8}+\tfrac{1}{8}s-\tfrac{1}{8}\lambda)$$
$$\equiv\Gamma(\tfrac{1}{8}\nu+\tfrac{1}{8}s+\tfrac{1}{16})\,\Gamma(\tfrac{1}{8}\nu+\tfrac{1}{8}s+\tfrac{5}{16})\,\Gamma(\tfrac{1}{8}\nu+\tfrac{1}{8}s+\tfrac{9}{16})\,\Gamma(\tfrac{1}{8}\nu+\tfrac{1}{8}s+\tfrac{13}{16})$$

is satisfied. Proceeding as before the equation is found to admit of three valid solutions, viz. the sets $(\frac{3}{2}, \frac{1}{2}, 1)$, $(\frac{1}{2}, \frac{1}{2}, 3)$ and $(\frac{1}{2}, \frac{3}{2}, 5)$ for the values of the parameters (λ, ρ, ν). These show that

$$x^{-\frac{1}{2}}e^{-x^2/2\sqrt{2}}\sin(x^2/2\sqrt{2}) \text{ is } R_1,\ x^{\frac{7}{2}}e^{-x^2/2\sqrt{2}}\sin(x^2/2\sqrt{2}) \text{ is } R_3 \text{ and}$$
$$x^{\frac{1}{2}}J_{\frac{3}{2}}(x^2/2\sqrt{2})\,K_{\frac{3}{2}}(x^2/2\sqrt{2}) \text{ is } R_5.$$

---

* G. N. Watson : loc. cit. §13.45 (1)

Result 3. Proceeding with the integral*

$$\int_0^\infty t^{s-1} J_\mu(\tfrac{1}{4}t^2)\, J_\rho(\tfrac{1}{4}t^2)\, dt = \frac{2^{\frac{3}{2}s-2}\,\Gamma(1-\frac{1}{2}s)\,\Gamma(\frac{1}{2}\mu+\frac{1}{2}\rho+\frac{1}{4}s)}{\Gamma(1+\frac{1}{2}\rho-\frac{1}{2}\mu-\frac{1}{4}s)\,\Gamma(1+\frac{1}{2}\rho+\frac{1}{2}\mu-\frac{1}{4}s)\,\Gamma(1+\frac{1}{2}\mu-\frac{1}{2}\rho-\frac{1}{4}s)},$$

$$R(\mu+\rho) > -\tfrac{1}{2}R(s) > -1,$$

as heretofore the functional equation† obtained is

$$\Gamma_2(\tfrac{3}{4}\pm\tfrac{1}{4}-\tfrac{1}{4}s-\tfrac{1}{4}\lambda)\;\Gamma_2(\tfrac{5}{8}\pm\tfrac{1}{4}+\tfrac{1}{4}\nu-\tfrac{1}{4}s)$$
$$\equiv\Gamma_2(1+\tfrac{1}{2}\rho\pm\tfrac{1}{2}\mu-\tfrac{1}{4}s-\tfrac{1}{4}\lambda)\;\Gamma(1+\tfrac{1}{2}\mu-\tfrac{1}{2}\rho-\tfrac{1}{4}s-\tfrac{1}{4}\lambda)\;\Gamma(\tfrac{1}{2}\mu+\tfrac{1}{2}\rho+\tfrac{1}{4}\lambda-\tfrac{1}{4}s+\tfrac{1}{4}).$$

This admits of eight solutions of which only three are found to be valid. These furnish the functions

$$x^{\frac{1}{2}} J_{-\frac{1}{4}}(\tfrac{1}{4}t^2)\, J_{\frac{1}{4}}(\tfrac{1}{4}t^2),\ x^{-\frac{5}{2}}\sin^2(\tfrac{1}{4}x^2) \text{ and } x^{-\frac{3}{2}}\sin(\tfrac{1}{4}x^2)\, J_{\frac{3}{2}}(\tfrac{1}{4}x^2)$$

which are respectively R_1, R_3, and R_5.

Result 4. Lastly, proceeding with the integral‡

$$\int_0^\infty t^{s-2\rho-1} H_\rho(\tfrac{1}{2}t^2)\,dt = \frac{2^{s-2\rho-2}\,\Gamma(\frac{1}{2}+\frac{1}{4}s)\,\Gamma(\frac{1}{2}-\frac{1}{4}s)}{\Gamma(\rho+1-\frac{1}{4}s)\,\Gamma(1-\frac{1}{4}s)},\quad 2>R(s)>-2,$$

we obtain finally the R_1 function

$$x^{\frac{1}{2}} H_{-\frac{1}{2}}(\tfrac{1}{2}x^2)$$

and the R_3 function $x^{-\frac{3}{2}}H_{\frac{1}{2}}(\frac{1}{2}x^2)\equiv x^{-\frac{5}{2}}\sin^2(\frac{1}{4}x^2)$ as in Result 3.

---

* G. N. Watson : loc. cit.

† For brevity the symbol Γ_2 is used to express $\Gamma(\lambda+\nu)\,\Gamma(\lambda-\nu)$ as $\Gamma_2(\lambda\pm\nu)$.

‡ G. N. Watson : loc. cit. § 13.24 (2).

Condensation of Benzaldehyde with Resacetophenone in the presence of Caustic Alkalies

By D. S. Mittal

(*Received February* 8, 1945)

Nadkarai, Saiyad and Wheeler (J. C. S. 1937, 1737) have discussed the various reports given by earlier workers with regard to the condensation of resacetophenone with benzaldehyde and protocatechualdehyde. They have also described the condensation of the two aldehydes brought about in the presence of alcohol and aqueous potassium hydroxide. The condensation of resacetophenone with benzaldehyde as described by Wheeler and collaborators (loc. cit) was repeated with some modification in the amount of potassium hydroxide and alcohol. The condensation proceeded exactly as described by them and their result and yield of 2 : 4 di-hydroxphenylstyryl-ketone were confirmed. They did not obtain, however, 7-hydroxy flavanone as a bi-product of this condensation but had obtained it by Ellia's, method (J. C. S. 1927, 1720) as well as by heating the chalkone with sodium hydroxide and also from 4-benzyloxy chalkone (Mahal, Rai, Venkataraman, J. C. S., 1935, 866).

In the experiments performed here and described below, the yield of chalkone was about 35 per cent. The chalkone was, also accompanied with flavanone (yield 2.21 %) which was separated from the chalkone by toluene, in which the flavanone was less soluble.

Experiment

A mixture of resacetophenone(5 grams), benzaldehyde (3.5 grams), potassium hydroxide (80 grams in 100 c.c. of water) and alcohol (50 c.c.) were mixed in a flat bottomed flask and the flask was shaken vigorously. An orange coloured solution which grew darker and darker, was obtained. The flask became hot during this time and so it was kept cool under tap water during this process of shaking. It was left aside for three days.

To a test portion dilute hydrochloric acid was added drop by drop until there was no further precipitate. Now the whole of the solution was diluted with water and extracted with ether to remove unchanged benzaldehyde. The chalkone was precipitated on the addition of dilute hydrochloric acid. First of all the chalkone was thrown down in the form of a dirty yellow oil which afterwards solidified. It was filtered, dried and weighed 2.6 grams (32.93 % yield). Another condensation using the same quantities of the reactants was carried out but the quantities of alcohol and water were interchanged, i.e., instead of using 50 c.c. of alcohol and 100 c.c. of water, 100 c.c. of

alcohol and 50 c.c. of water in this condensation were used and the above procedure was repeated. The chalkone was dried, and weighed 2.8 grams, 35.45 % yield. The product in both the cases was recrystallised from toluene in small yellow needles M.P. 150°C. (Ellison J. C. S. 1927, 1720, M.P. 133-134.0°.; Wheeler, and his collaborators J. C. S. 1937, M.P. 150°).

7-Hydroxyl-flavanone was not isolated completely when 5 grams of resacetophenone was used for condensation. It was only (0.7) grams (yield-2. 25 %) when 20 grams of resacetophenone was condensed with 14 grams of benzaldehyde.

This was separated from hot toluene in white needles from the crude product when all the 2 : 4-dihydroxyphenylstyryl-ketone was dissolved. It melted at 190-191°. This flavanone was also obtained from 2 : 4 dihydroxyl-phenylstyryl-ketone by the method of Wheeler and his collaborators (loc. cit.).

A mixed melting-point with the previous flavanone did not produce any depression in the melting point, and hence it was concluded that the flavanone always accompanied with the chalkone though in very small quantities. The method of preparation of the flavanone is simpler than that described by Wheeler and co-workers.

My personal thanks are due to Dr. Pandya for the kind help offered during the course of the above work and to the authorities of St. John's College, Agra, for the facilities offered for research.

Condensation of 3 : 5-Dinitro-2-Chlorobenzaldehyde with Malonic Acid in the presence of Organic Bases

By D. S. Mittal

(*Received February* 6, 1945).

The Condensation of 3 : 5 dinitro-4-chlorobenzaldehyde (Mittal, J. Ind., Chem., Soc., 1942, 19, 408) with malonic acid has already been studied (J. Ind., chem. Soc., 1944, 21, 34).

In this paper the condensation of 3 : 5-dinitro-2-chlorobenzaldehyde (J. Ind., Chem., Soc., 1942, 19, 408) has also been studied under the same conditions as has been previously observed in other cases (c.f. Mittal, J. Indian Chem., Soc., 1942, 19, 47).

In the experiments described below, i.e., in the presence of pyridine, piperidine, quinoline and without a base, the product obtained was only 3 : 5-dinitro-2-chloro-cinnamic acid, which, when a base was used, came out in 90 % yield.

Experimental

The condensations were carried out following the method of Pandya (J. Indian Chem., Soc., 1934, 11, 824, et, seq.,).

In all the experiments 2.30 gram of the aldehyde, 1.04 gram of malonic acid (pure for scientific purposes and dried) (1 : 1 mol. proportions) were heated on a water bath in a small round bottom flask with 0.15 mol. of the base for 4 hours. The whole mass became a homogeneous liquid after 45 min. and evolution of water vapour and carbon dioxide occured. It was then left overnight. The product in the flask was of a yellow colour. It was treated with 10 % sodium carbonate solution and filtered under suction. The product dissolved wholly when a base had been used but when it was not used, some insoluble unchanged aldehyde remained behind. The clear filtrate on acidification with dilute hydrochloric acid gave the required cinnamic acid, which was filtered, dried and weighed as it was fairly pure.

TABLE

	Catalyst	Amount of Catalyst	Yield	per cent yield
1.	Pyridine	0.15 mol.	1.08 gram	91.51 per cent
2.	Piperidine	,,	1.05 ,,	88.98 ,,
3.	Quinoline	,,	0.95 ,,	80.50 ,,
4.	Without a base	X	0.60 ,,	50.84 ,,

The cinnamic acid was recrystallised from alcohol in light yellow needles, m.p. 154-155°C.

Analysis.

1.	Nitrogen	a.	10.10 %
		b.	10.50 %
	Theory		10.27 %
2.	Chlorine	a.	13.25 %
		b.	13.23 %
	Theory		13.02 %

The author's thanks are due to Dr. Sane for guidance during the course of the work and to the authorities of the Lucknow University for facilities offered for Research.

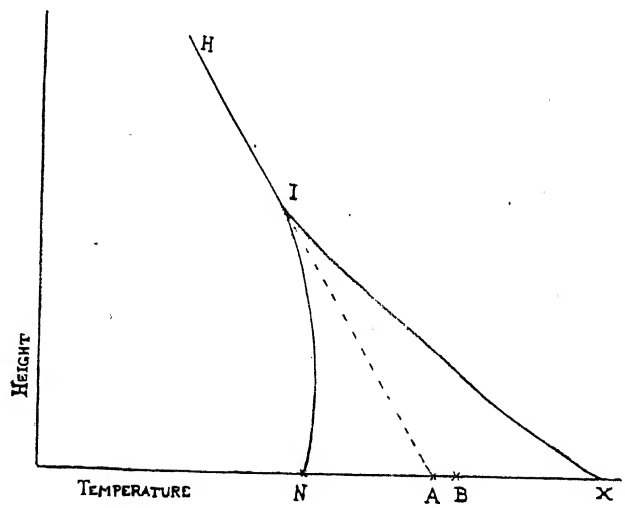
Identification of Air Masses in the Tropics and Surface Temperatures

By S. L. Malurkar

(Communicated by Professor K. S. Krishnan, F.R.S.—Received April 16, 1946).

Identification of air-masses is an important item in forecasting weather. Criteria have been developed which utilise upper air soundings (temperature and humidity at various levels). Several functions[1] of temperature and humidity have been used as being more or less conservative in the various meteorological transformations. However, at many places, a forecaster may have no access to the upper air soundings and may desire simpler criteria. In extra-tropical latitudes, the actual temperature and humidity at the surface itself give a fair indication of the air-masses. The difference in these elements between different air-masses is quite significant and detectable. But in the tropics, the temperature difference at the surface does not allow one to definitely distinguish the different air-masses. The problem arises whether there is any surface observation or observations involving temperature which can serve this purpose.[2]

In connection with problems on thunderstorms, many years ago, it was found that the specific humidity was a semi-conservative element in the absence of precipitation. The use of dew-point temperature has the same limitation.



Every air-mass has a more or less definite distribution of temperature and humidity with height. The freshly imported stream of such an air mass would show its characters at some height above ground. At ground surface, the distribution curve would be

appreciably affected by local factors, the chief of them being due to turbulence and radiation. These lead to large scale diurnal variations which may mask any actual difference in the air-mass temperatures. But above a certain height the effect of diurnal variations is small in comparision with the actual temperature or humidity differences of the air masses.

In the figure above (Temperature-Height curve) NIH is the curve at the time of minimum temperature and X I H at the time of maximum temperatures, above level I, the diurnal variation is negligible. The fanning out of the temperature element at lower levels is due to turbulence and radiation. If neither of these two factors existed, the temperature-height curve would have been A I H, often a linear extension of H I.

Hence what we need is a measure of the temperature given by the point I. It is not necessary to get the exact point I, it is sufficient if a quantity which is related to it is available. In the extra-tropical latitudes, attempts have been made to use the maximum temperature as a measure of the point. Others have tried to use the minimum temperature. In the tropics both these quantities would be very much affected by turbulence and radiation. If the temperature variation in a day was harmonic, the more obvious choice would be the mean temperature. It is well-known that in India and probably elsewhere in the tropics, the mean temperature bears a small constant difference with the average of the maximum and minimum temperature, when a period of 24 hrs is considered. The average temperature B of X and N may be displaced with reference to point A. For the same air-mass at about the same locality, it is natural to assume that the difference between A and B is constant. In practice, *the departure of mean temperature from normal at the same place and for the same day or period of the year* would be a useful criterion for each station. Any fresh incursion of air-mass would be quickly indicated by this factor. The importance of temperature departure has been stressed in the case of depressions and tropical cyclonic storms.[3]

The lapse-rate of temperature is greatly dependent on the moisture content. The dry continental air is dry-adiabatic and equatorial maritime air moist-adiabatic. The radiation from air layers is known to be dependent on the actual distribution of water-vapour with height. Hence the fanning out of the temperature curve at lower levels can itself be taken as a measure of the humidity distribution in the atmosphere to a limited extent. The diurnal range of temperature would be a measure of this fanning out. Here again, the problem of local effects has to be eliminated. For the same diurnal range of temperature, one place is overcast and the other has fine weather in the same season. A simple method of avoiding the local effect would be to take the *ratio of the diurnal range of temperature at a place to its normal value at that place for that day or particular period of the year*. When the diurnal range is more than usual, the air mass is drier than normal at the place and when the diurnal range is less than usual the air mass is more moist or maritime than normal. When the S. W. monsoon is strong, it is known that the

diurnal range of temperature at some hill stations becomes extra-ordinarily small.[4] Any fresh incursion of an air-mass should therefore be indicated by using the above two criteria.

Some attempts made to use the above criteria last year, were useful duringd isturbed periods. A systematic plotting of the above two values would facilitate the identification of incursion of fresh air-masses over a region.

References

1. See for example S. Petterssen. "Weather Analysis and Forecasting." p. 27.
2. The question was posed by Dewan Bahadur Ramanathan in a colloquium in June, 1945 at Poona Meteorological Office.
3. See. Forecasting Weather in and near India by author pp. 41, 90.
4. Ibid. p. 38. (Normand's criterion for Mahabaleswar).

New Aspects of Nitrogen Fixation in Soils and Origin of Soil Nitrogen

By N. R. Dhar

INTRODUCTION

It is interesting to note that the source from which plants take up their nitrogen was not definitely traced as late as the middle of the last century. Even the great chemist Liebig, whose writings deeply impressed agricultural science and practice in Europe did not realise the importance of supplying nitrogenous manure to soils as is evident from the following lines taken from his publication in Farmer's Magazine 1874. "If the soil be suitable, if it contains a sufficient amount of alkalies, phosphates and sulphates nothing will be wanting. The plants will derive their ammonia from the atmosphere as they do carbonic acid."

In the middle of the nineteenth century Boussingault suggested that vegetable earth contains certain living organisms some of which take part in the fixation of nitrogen in the soil. Inspired by Pasteur's researches, Jodin[1] (1862) first demonstrated that mycoderms growing in nitrogen free medium fixed nitrogen from the atmosphere. Later on Berthelot[2] (1885) announced that he obtained increases in the nitrogen content of normal but not sterilized soils. He further showed that the rise in the organic nitrogen content of soils left uncultivated for a period of several months is due to microbial activity. Hellriegel and Wilfarth[3] (1888) discovered the nodule organisms, Rhizobia, in the roots of leguminous plants and showed that these organisms, in association with the leguminous plants, bring about nitrogen fixation.

The above findings were soon followed by increasing evidence to show that nitrogen fixation in the soil was brought about by the activities of the micro-organisms present in the soil. Winogradsky[4] (1893) isolated a new anaerobic organism from the soil—Clostridium pasteurianum—which was found to fix nitrogen in the deeper layers of the soil. A more important discovery in this direction was that of Azotobacter chroococcum and Azotobacter agilis by Beijerinck[5] (1901). These organisms were isolated from soils and canal waters and found capable of nitrogen fixation.

It has been advocated that nitrogen fixation in soils is entirely a bacterial process although the amount of nitrogen fixed under non-symbiotic conditions is considered to be negligible as will be seen from the following quotations :—

" Laboratory investigations in humid climates suffer from the difficulty that the soils already contain so much nitrogen that small changes are difficult to measure accurately, and there are losses of nitrogen which counterbalance any fixation. Investigation would be easier in some soils, very poor in nitrogen found in hot, arid conditions.

Rigid incontestable proof could be furnished only by a demonstrated gain in nitrogen effected by Azotobacter, all other possibilities being ruled out. This proof is not yet forthcoming." (Russell, "Soil Conditions and Plant Growth," 1932, page 342.)

"It is difficult to obtain clear evidence showing how much nitrogen is fixed in soils in natural conditions, by free-living nitrogen-fixing organisms. Wherever a gain in nitrogen has been recorded in natural conditions in humid climate there have also been leguminous plants growing to which it might be attributed." (Russell, "Soil Conditions and Plant Growth," 1932, page 389).

"In view of the fact that the energy added to the soil is not directly available to the nitrogen-fixing bacteria, that small amounts of available nitrogen are always present in the soil, and the error in the laboratory determination by the Kjeldahl method is greater than the possible amount of nitrogen fixed by non-symbiotic bacteria, we are still unable to decide the question definitely," (Waksman, "Principles of Soil Microbiology," 1931, pages 514-515.)

"Wide use is being made in system of agriculture of the bacteria, which work with legumes, but the nitrogen fixing power of those which work outside the plant is as yet not utilised extensively by man, since the methods of controlling them are not well understood." (Miller, "The Soil and Its Management," 1934, page 203.)

For a number of years we 6_3 have been carrying on extensive research work on this phenomenon and we have established that nitrogen fixation can take place in soils when supplied with energy materials, like carbohydrates, glycerol, celluloses, cowdung, pentosans, fats, leaves, hay etc., and the amount of nitrogen fixed in light is always greater than in the dark and it is quite clear that in nitrogen fixation in soils sunlight or artificial light is utilised as in photosynthesis in plants. In nature large quantities of energy materials are added to the soil chiefly in the form of cellulose and the oxidation of this energy material leads to an enormous amount of nitrogen fixation, which is aided by sunlight. This is certainly the chief source of soil nitrogen and the nitrogen supply to the plants. This phenomenon appears to be in importance next to photosynthesis in plants. In artificial light exactly similar results have also been obtained.

The amount of nitrogen fixed in the soil on the addition of energy rich materials is always much greater than the amount fixed in the dark, though the number of micro-organisms is predominantly larger in the latter. The following results were obtained in fields and in dishes :—

NITROGEN FIXATION WITH CARBOHYDRATES

Field Trials.

Plot 4 feet by 4 feet containing 10 kilograms molasses (*i.e.* 25 tons of molasses per acre)

EXPOSED TO SUNLIGHT

Date.	NH_3-N%	NO_3-N%	Total Nitrogen%	Total Carbon%	Azotobacter per gram of dry soil in millions.	Total bacteria per gram of dry soil in millions.
13-2-37 (original soil)	0·0006	0·0014	0·0310	0·3472	0·9	12·0
9-3-37	0·0012	0·0016	0·0344	1·7708	12·5	38·0
26-4-37	0·0016	0·0016	0·0388	1·4136	75·0	245·0
12-7-37	0·0028	0·0016	0·0456	0·6874	155·0	385·0
25-9-37	0·0019	0·0017	0·0461	0·4728	115·0	305·0

Nitrogen fixed per gram. of carbon oxidized=8·9 mgm. (*i.e.* 338 lbs. fixed per acre in light.) ..

Covered with wooden planks (Dark)

13-2-1937 (original soil)	0·0006	0·0012	0 0300	0·3240	1·0	13·0
9-3-1937	0.0010	0·0015	0·0328	1·7732	16·0	48·0
16-4-1937	0.0013	0·0013	0·0344	1·4702	120·0	365·0
12-7-1937	0.0014	0·0015	0·0375	0·7854	290·0	615·0
25-9-1937	0.0013	0·0016	0·0388	0·4468	315·0	645·0

Nitrogen fixed per gram of carbon oxidized=3·56 mgm. (*i.e.* 197 lbs. fixed per acre in the dark).

Plot 4 feet by 4 feet containing 4 kilograms starch. (*i.e.* 10 tons of starch per acre).

EXPOSED TO SUNLIGHT

Date.	Total nitrogen%	Total Carbon%	Moisture	Azotobacter per gm. of dry soil in millions.	Total bacteria per gm. of dry soil in millions.
13-2-1937 (original soil)	0·0311	0·3374	1·5	1·5	13·5
12-3-1937	0·0333	1·0622	3·0	6·5	20·0
27-4-1937	0·0365	0·8618	4·0	48·0	140·0
24-5-1937	0·0388	0·7442	3·0	75·0	195·0
10-6-1937	0·0407	0·6702	3·5	70·0	210·0
11-7-1937	0·0424	0·5594	..	76·0	215·0
29-9-1937	0·0411	0·4684	4·0	35·0	175·0

Nitrogen fixed per gram of carbon oxidized = 16.5 mgm. (*i.e.* 253 lbs. nitrogen fixed per acre in light)

DARK

13–2–1937 (original soil)	0·0420	0·4360	1·5	1·5	13·5
12–3–1937	0·0437	1·1924	4·0	8·5	31·5
27–4–1937	0·0456	1·0214	4·0	70·0	205·0
24–5–1937	0·0462	0·9258	3·5	105·0	245·0
10–6–1937	0·0466	0·8205	4·5	130·0	282·3
11–7–1937	0·0472	0·7036	4·0	165·0	345·0
27–9–1937	0·0482	0·4864	4·8	162·6	350·8

Nitrogen fixed per gram of carbon oxidized = 5.9 mgm. (*i.e.* 138 lbs. nitrogen fixed in the dark)

Experiment in dishes.
1 kilogram soil + 20 gms. dextrin (temp. 34°-40°)
Exposed to Sunlight

Date.	Total Nitrogen %	Total Carbon %	Azotobacter per gram of dry soil in millions.
8–10–1936 (original soil)	0·0570	0·6156	5·2
10–12–1936	0·0608	1·1926	17·2
18–1–1937	0·0636	0·9414	28·5
4–2–1937	0·0646	0·7728	20·5
20–2–1937	0·0640	0·6292	18·5
6–3–1937	0·0636	0·6086	11·5

Nitrogen fixed per gram of carbon oxidized = 13.03 mgm.
Covered with black cloth.
Dark Temp (28°—31°)

8–10–1936 (original soil)	0·0570	0·6156	5·2
10–12–1936	0·0586	1·2644	32·5
18–1–1937	0·0600	1.1032	150·5
4–2–1937	0·0604	0·9778	198·5
20–2–1937	0·0608	0·8454	225·0
6–3–1937	0·0612	0·6868	280·0

Nitrogen fixed per gram of carbon oxidized =5·98 mgm.

1 Kilogram soil+20 gms. Fructose.

Exposed (Temp. 34°-42°)

Date.	Total Nitrogen %	Total Carbon %	Azotobacter per gram of dry soil in millions.
8–10–1936 (original)	0·0570	0·6156	5·2
25–10–1936	0·0570	1·3568	6·1
1–12–1936	0·0608	1·1518	19·8
19–1–1937	0·0646	0·7614	29·8
5–2–1937	0·0656	0·6346	23·0
7–3–1937	0·0626	0·6126	10·5

Nitrogen fixed per gram of carbon oxidized=11·9 mgm.

Dark (Temp. 28°-31°)

Date.	Total Nitrogen %	Total Carbon %	Azotobacter per gram of dry soil in millions.
8–10–1936 (original)	0·0570	0·6156	5·2
25–10–1936	0·0570	1·3745	7·2
1–12–1936	0·0590	1·2429	35·8
19–1–1937	0·0612	0·9876	225·5
22–2–1937	0·0618	0·7318	275·0
7–3–1937	0·0622	0·6126	290·0

Nitrogen fixed per gram of carbon oxidized =6·8 mgms.

From the foregoing results it is clear that although the number of Azotobacter and total bacteria in the dark is much larger than that in light, yet the amount of nitrogen fixation in soils, in plots as well as in dishes, is always nearly twice as great in light as in the dark. Moreover, the size of the Azotobacter colonies developed on plates containing the soil kept in the dark is much bigger than those obtained from the exposed ones. It appears, therefore, that the Azotobacter receiving sunlight is weakened. If bacterial metabolic activity is considered to go hand in hand with the growth activity, the fixation of atmospheric nitrogen in the dark basins or covered plots should have been more than in the ones exposed to light if no other agent was responsible in nitrogen fixation.

Summary of results obtained in dishes with carbohydrates and glycerol as energy materials.

Substance.	Nitrogen fixed per gram of carbon oxidized	
	Light.	*Dark.*
Glucose (2 per cent.)	12·5 mgm.	6·5 mgm.
Glycerol (5 per cent.)	6·04 mgm.	2·76 mgm.
Starch (5 per cent.)	7·58 mgm.	3·13 mgm.
Mannitol (2 per cent.)	12·8 mgm.	6·9 mgm.
Dextrin (2 per cent.)	13·03 mgm.	5·98 mgm.
Fructose (2 per cent.)	11·9 mgm.	6·8 mgm.
Maltose (2 per cent.)	12·6 mgm.	6·5 mgm.
Galactose (2 per cent.)	12·09 mgm.	6·7 mgm.
Field trials.		
Glucose (Plot 4 ft. by 4 ft.) containing 5 kilograms glucose.	14·0 mgm.	7·26 mgm.
Molasses (Plot 4 ft. by 4 ft.) containing 10 kilograms molasses.	8·9 mgm.	3·56 mgm.
Starch (Plot 4 ft. by 4 ft.) containing 4 kilograms starch).	16·5 mgm.	5·9 mgm.

In our field experiments we obtained the following results with molasses as a manure in increasing the soil nitrogen :—

Molasses added per acre.	*Nitrogen added per acre in light.*
3 tons	100 lbs (approximately).
10 tons	250 lbs. Do.
20 tons	350 lbs. Do.
30 tons	500 lbs. Do.

In the case of = starch and glycerol (5 per cent) and molasses (10 kg. in fields) the nitrogen fixed per gram of carbon is small when compared with others because of the high concentrations of the energy materials applied to the soil.

The following is the summary of results obtained at various temperatures on nitrogen fixation in the dark with soil and glucose kept at different temperatures in thermostats. The result of the corresponding set in sunlight is also recorded for comparison :—

Temperature.	Maximum number of Azotobacter per gram of dry soil in millions.	Nitrogen fixed in milligrams.
Exposed (42°)	22·5	13·1 mgm.
Dark (10°-12°)	6·0	nil.
Dark (25°)	126·0	4·8 mgm.
Dark (30°)	175·0	6·4 mgm.
Dark (35°)	200·0	7·76 mgm.
Dark (40°)	98·0	3·97 mgm.
Dark (45°)	78·0	3·03 mgm.
Dark (50°)	7·5	1·6 mgm.
Dark (60°)	nil.	nil.

From the foregoing results it is clear that the optimum temperature for nitrogen fixation in the dark is 35° as against 28° (25°-30°) observed in temperate countries. Above and below this temperature the fixation is less. At 11° and 60° the fixation is nil. The nitrogen fixed in the exposed soil, the temperature of which varied from 40° to 44°, is much greater than in those kept in the dark in the thermostats. Under comparable conditions, the nitrogen fixed per gram of carbon oxidized when the soil is exposed to sunlight is always greater than the nitrogen fixed per gram of carbon oxidized in the dark at various temperatures, whereas the Azotobacter numbers in the exposed soil, are much less when compared to those in the soils incubated at temperatures 25°, 30°, 35°, 40°, and 45°. In sunlight the nitrogen fixed is much greater than that obtained at the optimum temperature 35°. This definitely proves that the increase of temperature in sunlight is not at all the factor responsible for the greater nitrogen fixation observed but the increase in the nitrogen fixed in light is due to light absorption. Therefore, photochemical fixation of atmospheric nitrogen is very important and light plays a prominent role in fixation of nitrogen in soils.

Our field experiments as well as those carried on in dishes show that there is absolutely no denitrification even when large quantities of molasses, carbohydrates, glycerol etc., are added to soil exposed to air and light. The available nitrogen (sum of ammoniacal and nitric nitrogen) and the total nitrogen contents of the soil are never less than the original amounts present in the soil before the addition of energy materials. As a matter of fact, even a few days after the addition of molasses or carbohydrates to the soil, the ammoniacal nitrogen is appreciably increased and the available and total nitrogen contents also augment due to fixation of atmospheric nitrogen. When the amounts of molasses are large and the aeration of the soil inadequate, a part of the nitric nitrogen of the soil may be converted into ammoniacal nitrogen but it is never lost at the soil. The ammoniacal nitrogen, in course of time, is again oxidized to nitrate. This is a very important observation, which differs from the experience of workers in temperate climates, where marked decrease of available nitrogen has been observed on the addition of carbohydrates to soil.

Nitrogen Fixation with Increased Nitrogen Content of Soils

It is well known that the average status of nitrogen in Indian soils is about 0.04 to 0.05 per cent. and much smaller than that in the soils of temperate countries (about 0.08 to 0.1 per cent). I have, therefore, studied the effect of raising the nitrogen content of our soils to 0.8 per cent. in order to obtain results comparable with European soils on the efficiency of nitrogen fixation. It has been reported by previous workers like Burk (J. Bact. *19*, 400, 1930), Hills (J. Agric. Res. *12*, 183, 1918), and Bonazzi (J. Bact. *6*, 331, 1921) that in pure cultures the nitrogen fixing power of Azotobacter is completely inhibited by the presence of even 0.5 mgm. of available nitrogen per 100 c.c. medium solution. The following experiments have been carried with a view to find out how far the fixation of nitrogen is affected in soil by the presence of nitrogenous

Exposed.

Hours of exposure.	%Total carbon unoxidised gm.	%Total carbon oxidised gm.	%Total nitrogen mgm.	Loss of nitrogen%	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6
Zero	0·4764	..	80·00	..	1·7
75	0·4612	0·0152	76·08	4·90	..
175	0·4586	0·0178	70·12	12·35	..
275	0·4560	0·0204	61·76	22·80	2·8
		Dark.			
Zero	0·4764	..	80·00	..	1·7
75	0·4642	0·0122	77·60	3·00	..
175	0·4606	0·0158	73·82	7·74	..
275	0·4598	0·0166	72·24	9·70	6·2

(v) 50 *gm. soil*+0·0429 *gm. urea*+2·4045 *gm. sucrose*+20 *c.c. water* :—

Total carbon per 100 gm. soil .. .. 2·4000 gm.

Total nitrogen per 100 gm. soil .. .. 80·00 mgm.

C/N .. .. .. 30·0

Exposed.

Hours of exposure.	%Total carbon unoxidised gm.	%Total carbon oxidised gm.	%Total nitro-gen mgm.	%Total nitrogen gain mgm.	Efficiency.	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7
Zero	2·4000	..	80·00	..	..	1·7
75	2·1105	0·2895	82·16	2·16	7·47	..
175	1·9613	0·4387	83·09	3·09	7·05	..
275	1·8310	0·5690	83·82	3·82	6·72	10·3
			Dark			
Zero	2·4000	..	80·00	..	..	1·7
75	2·1614	0·2386	81·01	1·01	4·25	..
175	2·0392	0·3608	81·41	1·41	3·90	..
275	1·9295	0·4705	81·75	1·75	3·72	104·5

The foregoing results show that nitrogen fixation is possible when the soil nitrogen is as high as in some European soils (0.08%) but the efficiency is less than in the case of soils of tropical countries, although the amount of nitrogen fixed per gram of carbon oxidized is much greater in light than in the dark.

EXPERIMENTS WITH SALTS OF ORGANIC ACIDS AS ENERGY MATERIALS

Analysis of the original soil:—

Total carbon per cent.	0·4290
Total nitrogen per cent.	0·0405
pH	7·5
Azotobacter in million per gm. dry soil . .	3·5
Total carbon introduced per 100 gm. soil . .	0·80 gm.
Duration of exposure	From 1st May 1941 to 5th June 1941, 8 to 9 hours daily.
Temperature	48·5° to 55·6°

(i) 100 *gm. soil*+40 *c.c. water*+1.2676 *gm. sodium acetate*:—

Exposed.

Hours of exposure.	pH	%Total carbon unoxidised gm.	%Total carbon oxidised gm.	%Total nitrogen mgm.	Total Nitrogen gain mgm.	Efficiency.	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7	8
Zero	7·5	0·7983	..	40·50	..	..	3·5
100	9·3	0·6018	0·1965	41·96	1·46	7·45	..
200	..	0·5231	0·2752	42·45	1·95	7·10	..
300	9·4	0·4399	0·3584	42·87	2·37	6·62	4·9
			Dark.				
Zero	7·5	0·7990	..	40·50	..	..	3·5
100	9·2	0·6482	0·1508	41·10	0·60	3·98	..
200	..	0·5708	0·2282	41·35	0·85	3·74	..
300	0·2	0·5105	0·2885	41·51	1·01	3·50	9·7

(ii) 100 *gm. soil*+40 *c.c. water*+2.0714 *gm. sodium oxalate*:—

Exposed.

Zero	7·5	0·7976	..	40·40	..	..	3·5
100	9.1	0·6109	0·1867	41·79	1·39	7·45	..
200	..	0·5361	0·2615	42·24	1·84	7·02	..
300	9·1	0·4582	0·3394	42·63	2·23	6·55	4·8
			Dark.				
Zero	7·5	0·7970	..	40·40	..	..	3·5
100	9.1	0·6582	0·1388	40·94	0·54	3·90	..
200	..	0·5807	0·2163	41·19	0·79	3·65	..
300	9·1	0·5229	0·2741	41·36	0·96	3·45	9·7

(iii) 100 *gm. soil*+40 *c.c. water*+ 1.3292 *gm. sodium citrate* :—

Exposed.

Hours of exposure.	pH	%Total carbon unoxidised gm.	%Total carbon oxidised gm.	%Total nitrogen mgm.	Total nitrogen gain mgm.	Efficiency	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7	8
Zero	7·5	0·7974	..	40·40	..	..	3·5
100	8·8	0·6131	0·1843	41·94	1·54	8·38	..
200	..	0·5380	0·2594	42·50	2·10	8·08	..
300	8·8	0·4675	0·3299	42·95	2·55	7·72	6·8
				Dark.			
Zero	7·5	0·7982	..	40·50	..	..	3·5
100	8·7	0·6579	0·1403	41·11	0·61	4·35	..
200	..	0·5794	0·2188	41·42	0·92	4·20	..
300	8·8	0·5377	0·2605	41·56	1·06	4·05	18·2

(iv) 100 *gm. soil*+40 *c.c. water*+1.4958 *gm. sodium tartrate* :—

Exposed.

Hours of exposure.	pH	%Total carbon unoxidised gm.	%Total carbon oxidised. gm.	%Total nitrogen mgm.	Total nitrogen gain mgm.	Efficiency	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7	8
Zero	7·5	0·7992	..	40·50	..	..	3·5
100	9·3	0·6197	0·1795	41·87	1·37	7·65	..
200	..	0·5388	0·2604	42·40	1·90	7·28	..
300	9·4	0·4870	0·3122	42·59	2·09	6·70	5·3
				Dark.			
Zero	7·5	0·7988	..	40·50	..	..	3·5
100	9·2	0·6708	0·1280	41·03	0·53	4·16	..
200	..	0·5902	0·2086	41·32	0·82	3·95	..
300	9·2	0·5480	0·2508	41·42	0·92	3·66	11·2

(v) 100 *gm. soil*+40 *c.c. water*+1.1237 *gm. calcium lactate* :—

Hours of exposure.	pH	%Total carbon unoxidised gm.	%Total carbon oxidised gm.	%Total nitrogen mgm.	Total nitrogen gainmgm.	Efficiency	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7	8
Zero	7·5	0.7958	..	40·30	..	..	3·5
100	8·0	0.6437	0·1521	41·62	1·32	8·68	..
200	..	0.5570	0·2388	42·27	1·97	8·26	..
300	8·1	0.5002	0·2956	42·66	2·36	8·00	10·6
				Dark.			
Zero	7·5	0·7952	..	40·30	..	..	3·5
100	8·0	0·6766	0·1186	40·86	0·56	4·68	..
200	..	0·6035	0·1917	41·17	0·87	4·52	..
300	8·0	0·5492	0·2460	41·36	1·06	4·31	38·2

From the results recorded above it seems that alkalinity produced by the hydrolysis of the salts used as energy materials markedly lowers the efficiency of the process of nitrogen fixation, the fall being more marked in light than in the dark. The efficiency of fixation is comparatively larger with sodium citrate and calcium lactate, in both of which the pH is also less. It may be that some loss of nitrogen has taken place as ammonia on account of the alkalinity produced in the above set of experiments. But the amount of nitrogen fixed is again about twice greater in light than in the dark, though the number of Azotobacter is greater in the latter.

EXPERIMENTS IN STERILE CONDITIONS WITH CARBOHYDRATES AS ENERGY MATERIALS

We have also carried on experiments under completely sterile conditions and in such cases also we have obtained fixation of nitrogen not only with sterile soils but also with substances like ZnO, Fe_2O_3, Al_2O_3, MnO_2, CuO, CoO etc.

In the following experiments 50 grams soil were taken to which 1 gram glucose was added.

The experimental arrangements are the same as before and the results are as follows :—

(1) Soils in quartz flasks. The following experiments were started on 12-9-1938, and finally analyzed on 1-3-1939.

	Sunlight.		*Dark.*	
Original soil	Total nitrogen	0.0424%	Total Carbon	0.4305%
Substance	Total N.	Total C.	Total N.	Total C.
Inulin	0 0464%	0·7732%	0·0424%	0·9924%
Arabinose	0·0448%	0·7904%	0·0424%	0·9826%
Fructose	0·0464%	0·7635%	0·0432%	0·9786%
Lactose	0·0456%	0·7816%	0·0424%	0·9924%
Glucose	0·9456%	0·7635%	0·9432%	0·9562%
Mannitol	0·0456%	0·7732%	0·0424%	0·9826%
Glycerol	0·0448%	0·8048%	0·0424%	0·8968%
Galactose	0·0456%	0·7732%	0·0424%	0·0056%
Maltose	0·0464%	0·7735%	0·9424%	1·1026%
Dextrin	0·0464%	0·7732%	0·0432%	0·9826%
Starch	0·0448%	0·9264%	0·0424%	1·1206%
Control Soil	0·0408%	0·3986%	0·0416%	0·4208%

(2) Soils in Pyrex Flasks. Analyzed on 27-3-1939.

	Sunlight.		*Dark.*	
Substance	Total N.	Total C.	Total N.	Total C.
Inulin	0·0456%	0·8968%	0·0424%	0·1264%
Arabinose	0·0448%	0·9086%	0·0424%	1·0086%
Fructose	0·0448%	0·9156%	0·0432%	0·9924%
Glucose	0·0448%	0·8892%	0·0424%	0·9638%
Starch	0·0432%	1·1026%	0·0424%	1·1284%
Control soil	0·0416%	0·4208%	0·0416%	0·4208%

(3) Fixation with unsterile oxides. In these experiments 50 grams of oxide were mixed with 1 gram glucose. Experiments were started on 25-2-1940 and analyzed on 28-4-1940.

	Light.			*Dark.*		
	Total N.	Total C.	Total bacteria in million per gram.	Total N.	Total C.	Total bacteria in million per gram.
MnO_2	0·015 %	0·3128%	1·96	0·0058%	0·4286%	2·92
CuO	0·0088%	0·5092%	0·62	0·0027%	0·6498%	0·88
CoO	0·0187%	0·3035%	1·02	0·0085%	0·4012%	3·20
Ni_2O_3	0·0235%	0·2968%	0·98	0·010 %	0·3862%	2·98

Nitrogen fixed per gram of carbon oxidized.

	Light	*Dark*
MnO_2	30·78 mgms.	15·61 mgms.
CuO	30·26 ,,	17·97 ,,
CoO	37·66 ,,	21·31 ,,
Ni_2O_3	46·7 ,,	24·16 ,,

These nitrogen fixation results are very high and are being confirmed by further experiments.

(4) Nitrogen fixation with oxides of metals under sterile conditions (one litre Pyrex flasks used).

In the following experiments 25 grams of oxide were taken to which 0.5 gram glucose was added. Started on 13-2-1940 and analyzed on 1-9-1940.

Results obtained with sterile oxides

	Total N.	Total C.	N fixed per gram of carbon oxidized.
		Light.	
ZnO	0·0048%	0·4826%	15·12 mgms.
Al_2O_3	0·0038%	0·5348%	14·33 ,,
Fe_2O_3	0·0056%	0·5016%	18·76 ,,
Ni_2O_3	0·0056%	0·4972%	18·49 ,,
CoO	0·0048%	0·5124%	16·66 ,,
CuO	0·0020%	0·6628%	14·43 ,,
MnO_2	0·0048%	0·4624%	14·21 ,,
		Dark.	
ZnO	0·0017%	0·5892%	8·06 mgms.
Al_2O_3	0·0017%	0·6084%	8·87 ,,
Fe_2O_3	0·0020%	0·6172%	10·94 ,,
Ni_2O_3	0·0020%	0·6108%	10·57 ,,
CoO	0·0020%	0·6084%	10·43 ,,
MnO_2	0·0017%	0·6084%	8·87 ,,

In the foregoing cases the sterile experiments have been carried on in flasks plugged with sterile cotton wool while the unsterile experiments have been conducted in open enamelled dishes. It may be argued that the conditions of surface, exposure, moisture and most of all the conditions of oxidation are markedly different in the sterile and the unsterile sets. The results of oxidation and of nitrogen fixation under sterile condition may have diverged from the corresponding ones obtained in the unsterile state not only due to the presence of nitrogen fixing organisms in the latter but also on account of other differences in the experimental conditions of the sterile and unsterile sets. We have, therefore, investigated the fixation of nitrogen in soil on the addition of glucose as energy material under identical experimental conditions for both sterile and unsterile sets in light as well as in the dark. 50 gms. soil are mixed with 25 c.c. distilled water in one litre pyrex flasks. The sterile sets are then sterilised and 1 gm. glucose is added when they are again sterilised. 1 gm. glucose is also added to the corresponding unsterile sets which are also plugged with cotton wool so that the facility for oxidation may be the same in the sterile and unsterile sets. The plugs of the unsterile sets are, however, opened from time to time in order to promote bacterial contamination in them. One set of sterile and unsterile soils is exposed to the sun while the corresponding dark sets are covered with black cloth and placed beside

the exposed sets. All the flasks are shaken from time to time in order to facilitate aeration and to ensure the most uniform composition possible of the mixture of soil and glucose. Azotobacter counts have been made in the case of the unsterile sets while the sterile ones have been tested twice for any bacterial contamination and have been found to be perfectly free from it. The results of these experiments are as follows :—

Temperature—34° to 55°.

50 *grams soil*+1 *gm. glucose*+25 *c.c. water* :—

Exposed (unsterile).

Days of exposure.	%Total carbon (unoxidised) gm.	%Total carbon (oxidised) gm.	%Total nitrogen in milligram.	Total nitrogen gain in milligram.	Efficiency i.e. nitrogen fixed per gram of carbon oxidized.	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7
Zero	1·1796	..	32·20	..	..	2·8
25	1·0194	0·1602	34·09	2·09	13·05	..
45	0·8389	0·3407	36·53	4·33	12·72	6·2
65	0·7283	0·4513	37·71	4·51	12·20	..
85	0·6388	0·5408	38·45	6·25	11·55	2·6
			Dark (Unsterile).			
Zero	1·1796	..	32·20	..	..	2·8
25	1·0659	0·1137	32·91	0·71	6·20	..
45	0·9088	0·2708	33·80	1·60	5·92	32.5
65	0·7984	0·3812	34·36	2·16	5·66	..
85	0·6991	0·4805	34·82	2·62	5·45	167·2

Exposed (Sterile).

Days of exposure.	%Total carbon (unoxidised) gm.	%Total carbon oxidised gm.	%Total nitrogen mgm.	Total nitrogen gain mgm.	Efficiency.
1	2	3	4	5	6
Zero	1·1796	..	32·20	..	..
60	0·9535	0·2261	34·73	2·53	11·20
90	0·8412	0·3384	35·91	3·71	10·25
120	0·7589	0·4207	36·71	4·51	10·72
135	0·7311	0·4485	36·93	4·73	10·55
			Dark (Sterile).		
Zero	1·1796	..	32·20	..	..
60	1·0700	0·1096	32·73	0·53	4·85
90	0·9674	0·2192	33·20	1·00	4·72
120	0·8886	0·2910	33·54	1·34	4·60
135	0·8754	0·3643	33·55	1·35	4·45

Control soils kept under identical conditions.

	Light (*Unsterile*).			*Dark*		
Days of exposure.	%Total carbon (unoxidised) gm.	%Total nitrogen mgm.	Azotobacter count in million per gm. dry soil.	%Total carbon (unoxidised). gm.	%Total nitrogen mgm.	Azotobacter count in million per gm. dry soil.
1	2	3	4	5	6	7
Zero	0·3796	32·20	2·8	0 3796	32·20	2·8
45	0·3748	31·90	2·6	0·3750	32·90	2·8
85	0·3686	31·40	3·1	0·3725	32·60	3·1
			(*Sterile*).			
Zero	0·3796	32·20	..	0·3796	32·20	..
60	0·3756	31·90	..	0·3782	32·10	..
135	9·3706	31·50	..	0·3768	31·90	..

The following experiments were conducted under completely sterile conditions in pyrex glass vessels.

25 gms. oxide+0.5 gm. glucose+0.5 gm. V_2O_5 were exposed without any combined nitrogen to start with. Experiments started on 18-7-1940. The light set was analyzed on 2-12-1940 and the dark set on 28-1-1941.

	Light			*Dark*		
Experiment.	Total N.	Total C.	Nitrogen fixed per gram of C oxidized in mgm.	Total N.	Total C.	Nitrogen fixed per gram of C oxidised in mgm.
1	2	3	4	5	6	7
$ZnO+V_2O_5$	0·0056%	0·4628%	16·6	0·002%	0·5762%	8·47
$Fe_2O_3+V_2O_5$	0·0056%	0·4934%	18·5	0·0027%	0·5884%	9·40
$Al_2O_3+V_2O_5$	0·0048%	0·5086%	16·4	0·0017%	0·5912%	8·1
$MnO_2+V_2O_5$	0·0048%	0·4702%	14·8	0·0017%	0·6004%	8·5
$Ni_2O_3+V_2O_5$	0·0056%	0·4826%	17·6	0·002%	0·5884%	9·4

It is highly interesting to note that the nitrogen fixed per gram of carbon oxidized under completely sterile conditions in soils in quartz flasks is 12.2 mgms. in light and 4.8 mgms. in the dark.

Similarly, from the experiments carried on in pyrex glass vessels, which cuts off more light, especially the ultra-violet, than quartz, the mean nitrogen fixation is 11 mgms. per gram of carbon oxidized under perfectly sterile conditions in light, whilst in

the dark the fixation is 4·8 mgms. The order of these fixation under sterile conditions is practically the same as obtained without sterilization in soils.

It appears, therefore, that the efficiency of nitrogen fixation, whether the soil contains Azotobacter or is sterile, is practically the same, although the velocity of the oxidation of the energy materials is smaller in the sterile conditions than in the unsterile sets. In other words, the means by which the energy material is oxidized and the energy is made available does not materially affect the efficiency of the process.

When the energy materials are added to the soil, they are oxidized with the liberation of energy, and this energy is utilized in nitrogen fixation under ordinary conditions. In natural conditions the energy materials are oxidized on the soil surface and also oxidized by the living organisms. But under sterile conditions, the micro-organisms are destroyed and the phenomenon of oxidation is a non-biological surface and catalytic reaction. But the efficiency of the two processes, as far as nitrogen fixation is concerned, is of the same order in soils. Hence we are forced to conclude that nitrogen fixation can take place in as efficient a manner as possible in soils as well as in surfaces under sterile conditions in the same way as in natural conditions in the presence of living organisms.

From the foregoing results it is clear, that the amount of nitrogen fixation per gram of carbon oxidized with substances like ZnO, Al_2O_3, Fe_2O_3, Ni_2O_3, CoO, MnO_2, etc., with glucose as energy material is greater than with soil under comparative conditions, both in light and in the dark as well as in sterile and unsterile conditions. These results showing that nitrogen fixation is possible with chemically pure inorganic substances both in light and in the dark and that the efficiency of this process is greater with oxides than in soil are of fundamental importance. For nitrogen fixation neither soils nor bacteria are absolutely necessary, what really seems indispensable is a suitable surface where oxygen, nitrogen and an energy material are properly adsorbed and are in intimate contact. The energy material in contact with oxygen is oxidised on the surface and liberates energy necessary for the combination of nitrogen and oxygen. When light acts on the system a part of it is absorbed and causes a greater fixation of nitrogen. Hence in all cases the fixation of nitrogen is greater in light than in the dark.

We have observed that the nitrogen fixed in the form of protein, amino acid or ammonium salt does not remain in the combined state for a long time because the combined nitrogen has a tendency to undergo oxidation in air specially in light first into ammonia then into nitrite and finally into nitrate. In this process there is always the possibility of the formation and decomposition of the unstable substance ammonium nitrite. In the soil there is already a certain amount of combined nitrogen *i.e.* to the extent of 0.04 to 0.05% in tropical soils and hence this type of loss of nitrogen is likely to be more pronounced in soils than when oxide surfaces containing no nitrogen are used. Apparently the efficiency of the process of nitrogen fixation becomes better in presence of oxide surfaces than in soil containing nitrogen.

Probable Mechanism of Nitrogen Fixation

It is believed that under both anaerobic and aerobic conditions, ammonia is the first product of nitrogen fixation as ammonia is easily detected in the fixation of atmospheric nitrogen. Glucose has been found to decompose into pyruvic acid and hydrogen under anaerobic conditions according to the equation : $C_6H_{12}O_6 = 2CH_3COCOOH + 2H_2 + 12$ Cal. In the presence of the nitrogen of the atmosphere and on the soil surface, the hydrogen obtained from the decomposition of glucose may form ammonia according to the equation : $N_2 + 3H_2 = 2NH_3 + 24$ Cal.

In the presence of oxygen, however, that is, under aerobic conditions, it is difficult to assume that ammonia is also the first product of nitrogen fixation. Because, in the presence of oxygen, glucose can undergo one or more of the following oxidations on the soil surface :—

(1) $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 676$ Cal.
(2) $C_6H_{12}O_6 + 4\frac{1}{2}O_2 = 3C_2H_2O_4 + 3H_2O + 493$ Cal. Oxalic Acid.
(3) $C_6H_{12}O_6 + 1\frac{1}{2}O_2 = C_6H_8O_7 + 2H_2O + 199$ Cal. Citric Acid.
(4) $C_6H_{12}O_6 + O_2 = C_6H_{10}O_7 + H_2O + x$ Cal. Glycuronic Acid.
(5) $C_6H_{12}O_6 + \frac{1}{2}O_2 = C_6H_{12}O_7 + x$ Cal. Gluconic Acid.

It seems that in the presence of air, the first change (1) is the most important. The above organic acids, which may be produced under aerobic conditions in small quantities and the organic acids (*e.g.* acetic, propionic, butyric, lactic, etc.), and traces of alcohol glycerol, etc., which may be generated under anaerobic conditions are also easily oxidized to carbonic acid on the soil surface liberating energy. Hence, a large quantity of energy is available on the soil surface for nitrogen fixation on the addition of molasses or other energy materials.

In the soil containing compounds of iron, manganese, traces of titanium, copper, etc., which are excellent catalysts in oxidation reactions and exposed to sunlight and air, the oxidation of the energy materials is certainly due to bacteria, light and chemical catalysts all acting simultaneously. For obtaining the hydrogen required for ammonia formation, the following reaction has to take place:—$H_2O = H + OH - 112$ Cal. The direct combination of nitrogen and oxygen forming nitric oxide according to the equation :—$N_2 + O_2 = 2NO - 43{\cdot}2$ Cal. appears to require less energy than the process leading to the formation of ammonia from the atomic hydrogen obtained from water. It appears, that the iron compounds, traces of manganese, titanium and copper compounds and sunlight, which falls on the soil surface, can facilitate the formation of nitric oxide from oxygen and nitrogen of the air. The nitric oxide can be readily oxidized to nitrous and nitric acids, which form nitrates in the soil.

Dhar and Mukherjee have shown that solutions of nitrates and carbohydrates or glycerol in the presence of sunlight and titanium oxide can readily form small amounts of amino acids, with copious production of ammonium salts, whilst in the dark there

is no evidence of amino acid formation. It is interesting to note that ammonium salts and carbohydrates when exposed to sunlight in the presence of titanium oxide do not, however, form amino acids. Similarly cellulose and nitrates form small quant ies of amino acids in light in presence of titanium oxide. It is well known that in plants the proteins, which are the condensation products of amino acids, are only formed when carbohydrates have already accumulated by photosynthesis. The carbohydrates formed by photosynthesis in plants react with nitrates absorbed by plants from the soil and this results in the production of amino acids, proteins and ammonium salts in the plants. Recent work of Tottingham and Lowsma (1928) indicat s that the shorter visible light rays may greatly enhance the absorption of nitrate and significantly increase the synthesis of protein as evidenced by the early growth of wheat (compare Miller, Plant Physiology 1938, p. 618). As a matter of fact, Waynick add Woodhouse (California, Agric. Station Annual Report, 1918-1919, p. 62-63) have obtained evidence of amino acid formation in nitrogen fixation by Azotobacter. It is believed that in the first few days of the growth of Azotobacter amino acids accumulate and later on proteins increase. In our experiments with pure cultures of Azotobacter thriving in mannite we have been able to detect amino acids by the valuable 'ninhydrin' (triketo hydrindene hydrate) test in the filtered liquids obtained by crushing the Azotobacter cells with sand in a pestle and mortar. According to Jodidi[8], Schreiner and Skinner[9] and Lathrop[10] several amino acids are of common occurrence in the soil. These amino acids may be obtained either by the hydrolysis of proteins added to the soil as manure or formed synthetically as explained above. It seems likely, therefore, in vitro as well as in the plant and in the soil, the nitrate is reduced to ammonia by the action of carbohydrates or other carbonaceous compounds with simultaneous formation of amino acids in small quantities. Hence, it appears that nitrates are first produced in nitrogen fixation and the nitrates react with the energy rich-materials present in the soil with the formation of ammonium salts and small amounts of amino acids.

Although green plant is practically the sole converter of solar energy through the process of photosynthesis, it is a very inefficient machine, utilising only $0.50-3.0\%$ of the total energy falling upon it.

From our results of oxidation of energy materials and of nitrogen fixation we have calculated what fraction of the energy given out by the oxidation of energy materials is utilised in the process of nitrogen fixation, according to the energetics of the following equation :—

$$N_2+O_2=2NO-43.2 \text{ Cal.}$$

while the oxidation of 1 gm. of glucose (*i.e.* 72/180 gm. Carbon) liberates 4.1 Cal.

From the above energetics it has been calculated that 1 gm. carbon liberates 9.5 Cal. on oxidation, while the fixation of one milligram nitrogen absorbs only 0.00154 Cal. Therefore in the fixation of nitrogen only $0.08-0.10\%$ of the energy available is utilised in the dark while in light the corresponding value is 0.16 to 0.22. The

higher value in light is due to its absorption and utilization in nitrogen fixation. It may be pointed out here that in the Birkeland—Eyde process of nitrogen fixation too the efficiency is low, being only 1 to 2%. It appears, therefore that like the process of photosynthesis in plants the process of photochemical nitrogen fixation in soil is also inefficient. In a recent paper Dhar and co-workers (Proc. Nat. Inst. Sciences (India) 7 115, 1941) have stated that the increased production of nitric oxide in the arc process may be due to light absorption and its utilization by the system.

NITROGEN FIXATION WITH CELLULOSIC MATERIALS (HAY, LEAVES, COWDUNG AND PAPER)

The following lines from Waksman[11] show that the problem of the fixation of nitrogen in fields supplied with cellulosic materials has not yet been satisfactorily investigated.

"The importance of this process in increasing the supply of soil nitrogen is, however, still questionable." (Page 448).

"It has been found that polysaccharides like celluloses can also serve as valuable sources of energy if they are first partially broken down by cellulose-decomposing organisms. However, these results need still further confirmation." (Pages 561-562).

"Certainly the field results of A. Koch do not speak of any nitrogen fixation in the soil, following the addition of the celluloses and even straw". (Page 588).

Our results obtained with cellulosic materials are recorded below :—

1 Kilogram soil+20 grams filter paper. exposed (*i.e.*, 20 tons of filter paper per acre).

Date.	NH_3-N	NO_2-N	Total nitrogen.	Total carbon	Moisture.	Azotobacter per gram of dry soil in millions.
	%	%	%	%	%	
30-10-1936 (Original soil).	0·0011	0·0020	0·0540	0·567	2·2	2·4
22-12-1936	0·0008	0·0018	0·0540	..	3·8	3·7
20-1-1937	0·0007	0·0016	0·0560	..	3·1	7·7
20-3-1937	0·0006	0·0014	0·0583	..	3·0	12·5
7-5-1937	0·0006	0·0012	0·0646	..	3·5	20·5
7-6-1937	0·0006	0·0011	0·0677	..	3·1	27·2
8-7-1937	0·0007	0·0014	0·0666	0·7012	..	18·0
13-9-1937	0·0014	0·0021	0·0646	0·6704	..	12·0

Nitrogen fixed per gram of carbon oxidized=18.1 mgm.

(*i.e.* 306 lbs. of nitrogen fixed per acre of soil in light).

1 Kilogram soil+20 grams Filter paper. dark. (*i.e.* 20 tons of filter paper per acre).

Date.	NH_3-N	NO_3-N	Total nitrogen.	Total Carbon.	Moisture.	Azotobacter per gram of dry soil in millions.
	%	%	%	%	%	
30-10-1936 (Original soil).	0·0011	0·0020	0·0540	0·5670	2·2	2·4
22-12-1936	0·0007	0·0015	0·0540	..	4·8	4·3
20-1-1937	0·0006	0·0012	0·0540	..	4·2	5·7
20-3-1937	0·0006	0·0010	0·0552	..	4·0	25·5
7-5-1937	0·0006	0·0009	0·0567	..	3·0	60·0
7-6-1937	0·0006	0·0009	0·0575	..	..	80·0
8-7-1937	0·0006	0·0009	0·0583	..	..	92·5
13-9-1937	0·0008	0·0001	0·0608	0·6486	..	145·0

Nitrogen fixed per gram of carbon oxidized 9·2 mgm. (*i.e.* 152 lbs. nitrogen fixed per acre in the dark).

Plot 4 ft. by 4 ft. containing 20 kilograms wet cowdung. Exposed. (*i.e.* 50 tons of wet cowdung per acre).

Date.	Total nitrogen.	Total carbon.	Moisture.	Total Azotobacter per gram of dry soil in millions.	Total bacteria per gram of dry soil in millions.	Fungi per gram of dry soil.
(Original soil)	%	%	%	%	%	
10-2-1937	0·0323	0·3294	1·7	1·05	10·7	22·000
12-2-1937	0·0356	0·7126	..	..	..	..
7-3-1937	0·0368	0·6384	3·0	6·5	20·5	32·000
5-4-1937	0·0388	0·5616	3·5	15·5	65·5	41·000
29-4-1937	0·0424	0·4826	3·0	32·0	160·0	30·000
25-5-1937	0·0442	0·4108	3·5	30·0	170·0	28·000
12-6-1937	0·0466	0·3825	3·5	28·5	165·5	28·000
28-9-1937	0·0446	0·3789	3·2	15·8	115·2	25·000

Nitrogen fixed per gram of carbon oxidized=33·3 mgm. (*i.e.* nitrogen added in the cowdung=73 lbs. per acre but fixed in light=246 lbs. per acre).

Plot 4 ft. by 4 ft. containing 20 kilograms cowdung., covered *i.e.* (50 tons of wet cowdung per acre).

Date.	Total Nitrogen.	Total Carbon.	Moisture.	Azotobacter per gram of dry soil in millions.	Total bacteria per gram of dry soil in millions.	Fungi per gram of soil.
Original soil)	%	%	%			
10-2-1937	0·0356	0·3987	1·8	1·15	11·2	23·000
12-2-1937	0·0381	0·7218	..	..	..	..
7-3-1937	0·0385	0·6586	4·0	7·5	26·5	40·000
5-4-1937	0·0392	0·5944	4·0	21·5	112·0	56·000
29-4-1937	0·0403	0·5168	3·5	48·5	270·0	59·000
25-5-1937	0·0411	0·4684	4·0	55·0	315·0	48·000
12-6-1937	0·0420	0·4158	4·5	65·5	335·0	46·000
28-9-1937	0·0428	0·4012	5·0	49·5	300·0	41·000

Nitrogen fixed per gram of carbon oxidized=14·6 mgm. (*i.e.* nitrogen added in the cow dung = 56 lbs per acre but fixed in the dark=105 lbs. per acre).

Plot 4 ft. by 4 ft. containing 5 kilograms of hay. exposed. (*i.e.*, 12.5 tons of hay per acre of land).

Date.	T.N. %	T.C. %	Moisture %	Azotobacter per gram of dry soil in millions.	Total bacteria per gram of dry soil in millions.	Fungi per gram of dry soil.
22-1-1937 (Original soil).	0·0323	0·3488	1·7	1·58	12·7	27600
3-3-1937	0·0350	..	3·0	10·5	25·0	38000
7-4-1937	0·0381	..	3·5	20·5	85·5	36000
5-5-1937	0·0417	..	3·0	43·5	177·5	33000
29-5-1937	0·0442	..	3·5	55·5	220·0	30000
15-6-1937	0·0466	..	3·0	45·0	129·0	28000
23-9-1937	0·0512	0·6724	4·0	38·5	225·0	29000
28-10-1937	0·0500	0·5736	4·5	21·5	195·5	26000

Nitrogen fixed in light=423 lbs. per acre.

Plot 4 ft. by 4 ft. containing 5 kilograms of hay. covered. (*i.e.* 12.5 tons of hay per acre).

22-1-1937	0·0442	0·4199	1·8	1·14	13·3	25100
3-3-1937	0·0456	..	4·0	18·0	32·0	43000
7-4-1937	0·0472	..	4·5	35·5	140·5	48000
5-5-1937	0·0491	..	4·0	55·0	231·0	44000
29-5-1937	0·0512	..	4·0	70·0	300·0	46000
15-6-1937	0·0525	..	..	82·0	360·0	40000
23-9-1937	0·0560	0·7854	5·2	92·8	38 ·0	41000
28-10-1937	0·0560	0·6459	4·8	75·3	315·6	36000

Nitrogen fixed in the dark=264 lbs. per acre.

The foregoing results showing marked nitrogen fixation in soils with cellulosic materials are of considerable importance as celluloses and hemicelluloses form the major part of plant materials added to the soil. In this connection, the following lines from "Nature" (Feb. 1, 1941) are of interest:—"So far only Vartiovaara seems to have given indisputable proof of nitrogen fixation in combined cultures of one nitrogen fixing and one cellulose decomposing organism. Experiments by Jenson and Swaby have shown that no nitrogen is fixed when Azotobacter is grown in association with pure cultures of typical aerobic cellulose decomposing bacteria (cytophaga, callvibrio etc.) as well as fungi and actinomycetes". The counts of Azotobacter, total bacteria and fungi as recorded above and in the following pages show that the numbers of micro-organisms are less in light than in the dark but the nitrogen fixation in light is much greater than in the dark.

Moreover, the Woburn experiments with the dung obtained by feeding decorticated cotton cake, containing 6.6% nitrogen and maize-meal, with 1.7% nitrogen did not reveal any superiority of the cake-feeding over corn feeding in the manuring of wheat and barley. These observations have not yet been explained but it appears to be clear from the fact that the dung of maize-meal containing smaller amounts of initial total nitrogen fixes atmospheric nitrogen and makes up for its deficiency of total nitrogen due to the greater percentage of carbon in the maize-meal and its oxidation.

THE FOLLOWING EXPERIMENTS WERE CARRIED ON WITH LEAVES AS ENERGY MATERIALS

(a) 100 grams of soil+4 grams of dry powdered Neem (Melia Azadiractta Lina) leaf

(A) Exposed to Sunlight.

Number of hours exposed.	Moisture percentage.	Total carbon present in 100 grams of soil in grams.	Total carbon Oxidized per 100 grams of soil in grams.	Total nitrogen present in 100 gms. of soil in mgms.	Gain in nitrogen per 100 grams of soil in mgms.	Efficiency.	Azotobacter in millions per gram of dry soil.	Total bacteria in millions per gm. of dry soil.	Fungi per gram of dry soil.	pH.
Zero	1·0	1·6826	..	80·20	..	..	5.6	24·0	15000	7·5
150	1·8	1·2900	0·3926	87·60	7·4	18·84	19·5	40·0	18000	
300	2·4	1·1755	0·5071	89·70	9·5	18·73	32·0	56·2	24000	
450	2·9	1·0784	0·6042	91·50	11·3	18·70	54·8	78·4	26000	
600	3·2	1·0654	0·6172	91·80	11·6	18·79	67·0	92·0	23000	
800	3·0	0·9504	0·7322	91·40	..	..	60·0	84·0	20000	
1000	3·1	0·9108	0·7718	91·00	..	..	54·3	80·1	18000	
1250	2·8	0·8874	0·8052	89·20	..	..	41·5	67·2	14000	
				(B) Covered with Black Cloth.						
Zero	1·0	1·6630	..	80·00	..	..	5·0	25·0	15000	7·5
150	2·0	1·3425	0·3205	82·80	2·8	8·73	25·0	62·8	20000	
300	2·8	1·2549	0·4081	83·60	3·6	8·82	180·4	210·0	25000	
450	3·5	1·1316	0·5314	84·70	4·7	8·84	220.0	268·4	32000	
600	3·5	1·0023	0·6607	85·80	5·8	8·77	254.0	389·5	32000	
800	2·8	0·9821	0·6809	85·50	..	..	250.0	380·0	30000	
1000	2·8	0·9641	0·6989	85·30	..	..	247.8	375·0	26000	
1250	3·0	0·9305	0·7325	84·10	..	..	212.0	350·0	21000	

(b) 100 grams of soil +8 grams of dry Neem Leaf

(A) Exposed to Sunlight

Number of hours exposed.	Moisture percentage.	Total Carbon present in 100 grams of soil in grams.	Total Carbon Oxidized per 100 grams of soil in grams.	Total nitrogen present in 100 gms. of soil in mgms.	Gain in nitrogen per 100 grams of soil in mgms.	Efficiency.	Azotobacter in millions per gram of dry soil.	Total bacteria in millions per gm. of dry soil.	Fungi per gram of dry soil.	pH.
Zero	1·0	3·2970	..	116·00	..	..	5·8	23·6	15000	7·5
150	2·0	2·3773	0·9197	127·80	11·80	12·83	23·4	52·7	19000	
300	2·6	1·7327	1·3643	135·60	19·60	12·52	44·4	69·0	24000	
450	3·5	1·6012	1·6958	137·00	21·00	12·38	92·0	115·2	28000	
600	3·5	1·4902	1·8068	138·50	22·50	12·45	120·0	154·5	26000	
800	3·8	1·4001	1·8969	138·10	..	..	114·8	150·0	25000	
1000	3·4	1·3844	1·9126	137·40	..	..	108·4	141·0	20000	
1250	3·0	1·0908	2·2062	134·50	..	..	92·0	123·0	14000	
			(B) Covered with Black Cloth.							
Zero	0·9	3·2400	..	116·20	..	..	6·2	24·5	15000	7·5
150	2·5	2·4455	0·7945	121·40	5·20	6·54	52·4	84·2	21000	
300	3·0	2·0200	1·2200	124·10	7·90	6·47	250·6	290·0	30000	
450	3·6	1·9405	1·2995	124·40	8·20	6·31	325·0	376·4	32000	
600	3·5	1·8981	1·3419	125·00	8·80	6·55	367·4	408·0	33000	
800	3·2	1·8102	1·4298	124·70	..	..	351·0	382·0	30000	
1000	2·8	1·7651	1·4749	124·20	..	..	332·0	375·0	17000	
1250	3·2	1·5809	1·6591	122·80	..	..	264·0	345·0	21000	

(c) 100 grams of soil+12 grams of Neem leaf dry and powdered

(A) Exposed to Sunlight

F 6

Number of hours exposed.	Moisture percentage	Total Carbon present in 100 grams of soil in grams.	Total Carbon oxidized per 100 grams of soil in grams.	Total nitrogen present in 100 gms. of soil in mgms.	Gain in nitrogen per 100 grams of soil in mgms.	Efficiency.	Azotobacter in millions per gram of dry soil.	Total bacteria in millions per gm. of dry soil.	Fungi per gram of dry soil.	pH.
Zero	1·0	4·1200	..	150·0	..	..	5·5	24·2	15000	7.5
150	2·4	3·6730	0·4470	154·2	4·2	9·39	30·5	75·0	20000	
300	2·8	3·0060	1·1140	160·2	10·2	9·15	58·4	92·0	25000	
450	3·1	2·9020	1·2180	161·6	11·6	9·06	110·0	162·0	28000	
600	3·5	2·8001	1·3199	162·2	12·2	9·24	145·0	184·0	27000	
800	3·5	2·7304	1·3896	161·8	..	..	138·5	180·0	25000	
1000	3·0	2·6743	1·4457	160·7	..	..	126·0	164·8	21000	
1250	3·2	2·3578	1·7622	158·3	..	..	105·0	140·0	15000	

(B) Covered with Black Cloth.

Zero	1·0	4·1286	..	151·5	..	..	6·2	24·5	15000	7.5
150	2·5	3·8000	0·3286	152·9	1·4	3·26	60·5	98·5	25000	
300	3·0	3·3453	0·7833	154·7	3·2	4·08	285·4	305·0	28000	
450	3·5	3·1360	0·9926	155·5	4·0	4·03	386·5	456·0	33000	
600	3·5	3·0060	1·1226	156·0	4·5	4·00	445·0	525·0	35000	
800	3·4	2·9124	1·2162	155·6	..	..	400·0	492·0	29000	
1000	3·3	2·6450	1·4836	154·8	..	..	372·0	410·0	25000	
1250	3·5	2·4508	1·6778	153·0	..	..	340·0	371·0	20000	

Experiments with Cowdung under Sterile Conditions

Analysis of fresh cow-dung :—

Total carbon per cent. 8 6205

Total nitrogen per cent. 0.3801

Total carbon introduced per 100 gm. soil in cow-dung set. 0.3966+0.7759=1.1725 gm.

Total nitrogen introduced in the cow-dung set per 100 gm. soil. 33.60+34.20=67.80 mgm.

Duration of exposure of cow-dung set in quartz flask (sterile). From 23rd August, 1940 to 19th February, 1941, 8 hours daily.

50 grams soil+4.5 gm. fresh cow-dung+30 c.c. water + 1 gram of the catalyst.

Exposed (sterile).

Energy materials & catalysts.	% T.C. (unoxidised). gm.	% T.C. oxidised gm.	% T.N. mgm.	gain T.N. mgm.	Efficiency
1	2	3	4	5	6
1. Soil+Cow-dung+TiO_2+ water.	0 8603	0·3122	70·90	3·10	9·93
2. Soil+Cow-dung+V_2O_5+ water.	0 8729	0·2996	70·60	2·80	9·35
3. Soil+Cow-dung+mixture+ water.	0·8517	0·3208	70·80	3·00	9·38
4. Soil+Cow-dung+water–	0·8956	0·2769	70·20	2·40	8·66
5. Control (Soil+water)	0·3848	..	32·70	..	..
Dark (sterile)					
1. Soil+Cow-dung+TiO_2+ water.	·9667	0·2058	68·80	1·00	4·86
2. Soil+Cow-dung+V_2O_5+ water.	0·9795	0·1930	68·70	0·90	4·61
3. Soil+Cow-dung+mixture+ water.	0·9552	0·2173	68·70	0·90	4·14
4. Soil+Cow-dung+water...	1·0111	0·1614	68·40	0·60	3·72
5. Control (soil+water) ..	0·3906	..	33·30	..	..

Our results obtained under unsterile conditions show that the nitrogen fixation per gram of carbon in the cow-dung oxidized varies from 12.8 mgms. to 11.6 mgms. in light and 6.3 to 5.6 mgms. in the dark. Hence on comparison with the results obtained

under sterile conditions as recorded in the above tables, it appears that the nitrogen fixation under sterile conditions is slightly less than under unsterile conditions, the difference is likely to be due to the protein present in the micro-organisms present under unsterile conditions.

EXPERIMENTS WITH LEAF UNDER STERILE CONDITIONS

Analysis af fresh leaf:—

Total carbon per cent. 23·59

Total nitrogen per cent. 0·820

Total carbon reduced per gm. soil in the leaf set. 0·3966+0·9436=1·3402 gm.

Total nitrogen introduced per 100 gms. soil in the leaf set. 33·60+32·80=66·40 mgms.

Duration of exposure of leaf in the sterile set in quartz glass flask. From 17th August, 1940 to 18th March, 1941, 8 hours daily.

50 gm. soil +2 gm. fresh leaf+30 c.c. water+1 gram of the catalyst.

EXPOSED UNDER STERILE CONDITIONS

	Energy materials and catalysts.	%Total Carbon un-oxidized.	%Total Carbon oxidized.	%Total nitrogen.	Gain in nitrogen.	Efficiency.
	1	2	3	4	5	6
1.	Soil+leaves+TiO_2+water	1·0475	0·2927	69·10	2·70	9·24
2.	Soil+leaves+V_2O_5 +water	1·0666	0·2736	69·00	2·60	9·50
3.	Soil+leaves+mixture+water	1·0249	0·3153	69·00	2·60	8·24
4.	Soil+leaves+water ..	1·1061	0·2341	68·30	1·90	8·11
5.	Control (soil+water) ..	0·3805	..	32·70	..	..

Dark Sterile

	Energy materials and catalysts.	%T. C. (unoxidised) gm.	%T. C. oxidised gm.	%T. N. mgm.	Gain T. N. mgm.	Efficiency
	1	2	3	4	5	6
1.	Soil+leaves+TiO_2+water	1·1530	0·1872	67·30	0·90	4·80
2.	Soil+leaves +V_2O_5+water	1·1693	0·1709	67·20	0·80	4·68
3.	Soil+leaves+mixture+ water.	1·1314	0·2088	67·20	0·80	3·83
4.	Soil+leaves+water ..	1·2185	0·1217	66·90	0·50	4·14
5.	Control (soil+water) ..	0·3897	..	33·20	..	..

Our results show that the nitrogen fixation per gram of carbon of the leaf oxidized varies from 10·25 mgms. to 9·3 mgms. in light and 5·6 to 4·6 mgms. in the dark

under unsterile conditions. Hence the order of efficiency with leaf as energy material both under sterile and unsterile conditions is about the same .

These results obtained with cow-dung and leaf as energy materials under completely sterile conditions are in agreement with those obtained with carbohydrates and soil under the same conditions. The efficiency of nitrogen fixation both under sterile and unsterile conditions is about the same and hence the mechanism of the process seems to be identical in sterile as in unsterile conditions. These conclusions are of fundamental importance and far reaching consequence.

The for going results show that the cellulosic substances like filter paper, hay, leaf etc., when mixed with soil and exposed to sunlight or kept in the dark or diffused light cause nitrogen fixation. The nitrogen fixation in sunlight is greater than in the diffused light or in the dark. Similar results have been obtained with cow-dung. These results are most important because they show that cellulosic materials, plant residues, leaves, cow-dung, etc., not only increase the humus content of the soil, and improve the soil tilth, moisture retention capacity but also act in the increase of the soil fertility by nitrogen fixation. Hence cow-dung, which is used as a manure has been found to supply to the soil not only the nitrogen it contains but it can also add nitrogen to the soil from the nitrogen of the air by fixation.

The results obtained with soil and leaves as energy materials show clearly that the amount of nitrogen fixed per gram of carbon oxidized falls off with the increase in the total amount of nitrogen originally present in the mixture. This is a general phenomenon and is due to the fact that along with nitrogen fixation there is the concomitant process of nitrogen loss associated with the oxidation of nitrogen compounds present in the mixture or formed in the nitrogen fixation.

Schroder computes that roughly 35 billion kilograms of cellulose are added to he earth every year. From our experiments on nitrogen fixation with cellulosic materials we find that about 18 mgms. of nitrogen are fixed per gram of carbon oxidized from filter paper and leaves in light and about 9 mgms. in dark. While with cow-dung the corresponding results are respectively 33 mgms. and 14 mgms. in light and dark.

Hence, from the 35 billion k. gms. of cellulose added to the soil on a moderate estimate of 10 mgms. of average nitrogen fixation per gram of carbon oxidized, about 13,000,000 metric tons of nitrogen are added to the earth by fixation (the total output of N fixed synthetically in the world was 3,547,352 tons in 1937).

From our experiments we can conclude that out of the total 13,000,000 metric tons at least 50 per cent *i.e.* 6,500,000 metric tons of nitrogen are fixed in soils by the absorption of solar light.

It appears, therefore, that such more nitrogen is fixed in nature by light absorption than that fixed by the industrial processes. Hence, in the economy of nature, next to photosynthesis in plants, the inufluence of light on nitrogen fixation leads to incalculable benefit to plants and animals and is of very considerable value.

In a very recent paper (Smith, Wheeting and Vandecaveye, Soil Science, 1946, 61, 393), have reported nitrogen fixations of 38 lbs. per acre using 1·425 ton of straw and 76 lbs. per acre applying 3·125 tons of manure per acre in alternate years in the semi-arid soils, Washington U.S.A. Moreover, the observations of Howard and Wad (Waste Products of agriculture 1931, p. 100) show that a certain amount of nitrogen fixation also take -5 place in the composting of the waste products of agriculture, when the aeration of the compost heaps is adequate.

Fat as Energy Material in Nitrogen Fixation

We have used both butter and ghee (clarified butter) as energy materials in nitrogen fixation. We have observed that the oxidation of these substances when mixed with soil is slower than the oxidation of carbohydrates and even that of cellulosic materials.

The following results have been obtained in dishes, as well as in fields :—

1 Kilogram soil+20 grams butter.

(*Exposed*)

Date.	NH_3-N	NO_3-N	Total Nitrogen	Total Carbon	Moisture	Azotobacter per gram of dry soil in millions.
	%	%	%	%	%	
13-10-1936 (Original soil).	0·0014	0·0032	0·0570	0·6156	1·8	5·1
14-11-1936 ..	0·0015	0·0032	0·0570	1·4195	2·6	5·8
15-12-1936 ..	0·0016	0·0032	0·0570	1·3883	3·1	7·6
13-1-1937 ..	0·0014	0·0029	0·0570	1·3497	3·5	9·5
18-2-1937 ..	0·0009	0·0024	0·0591	1·1968	3·0	12·0
11-5-1937 ..	0·0007	0·0011	0·0617	0·9381	3·0	35·0
11-9-1937 ..	0·0006	0·0010	0·0646	0·6654	3·0	25·0
12-10-1937 ..	0·0009	0·0011	0·0626	0·6318	3·2	15·6

Nitrogen fixed per gram of carbon oxidized=10.07 mgm.

1 kilogram soil+20 grams butter.

(*Dark*)

Date.	NH_3-N	NO_3-N	Total Nitrogen	Total Carbon	Moisture	Azotobacter per gram of dry soil in millions.
	%	%	%	%	%	
13-10-1936 (Original soil).	0·0014	0·0032	0·0570	0·6156	1·8	5·1
14-11-1936 ..	0·0014	0·0032	0·0570	1·4564	3·9	6·6
15-12-1937 ..	0·0015	0·0030	0·0570	1·4278	4·0	8·5
13-1-1937 ..	0·0002	0·0028	0·0570	1·3962	4·4	14·6
18-2-1937 ..	0·0007	0·0021	0·0583	1·2854	4·0	20·0
11-5-1937 ..	0·0006	0·0010	0·0591	1·0952	4·0	50·0
11-9-1937 ..	0·0006	0·0009	0·0600	0·7456	3·5	70·0
12-10-1937 ..	0.0007	0.0009	0·0591	0·6036	3·8	58·8

Nitrogen fixed per gram of carbon oxidized=4.22 mgm.

Plot 4 ft. by 4 ft. containing 2 kilograms of Ghee (clarified butter) (*i.e.* 5 tons of ghee per acre of land).

(*Exposed*)

Date.	Total Nitrogen.	Total Carbon.	Moisture.	Azotobacter per gram of dry soil in millions.	Total bacteria per gram of dry soil in millions.	Fungi per gram of soil.
	%	%	%			
26-1-1937 (Original soil).	0·0368	0·3901	1·56	1·35	14·5	29·000
28-1-1937 ..	0·0368	1·0797	..	..	..	..
6-4-1937 ..	0·0381	0·9876	2·5	7·5	32·5	38·000
4-5-1937 ..	0·0392	0·8873	3·0	20·0	95·5	36·000
26-5-1937 ..	0·0400	0·8136	3·5	46·5	160·5	30·000
14-6-1937 ..	0·0407	0·7194	..	40·0	196·0	33·000
22-9-1937 ..	0·0439	0·4528	3·3	38·4	205·8	28·000
27-10-1937 ..	0·0420	0·4318	4·8	20·6	175·6	27·000

Nitrogen fixed per gram of carbon oxidized=11.0 mgm. *i.e.* 154 lbs. nitrogen fixed per acre in light.

Plot 4 ft. by 4 ft. containing 2 kilograms of Ghee (clarified butter).

(*Covered*)

Date.	Total Nitrogen.	Total Carbon.	Moisture.	Azotobacter per gramof dry soil in millions.	Total bacteria per gram of dry soil in millions.	Fungi per gram of soil.
	%	%	%			
26-1-1937 (original soil).	0·0381	0·4115	1·6	1·4	13·6	28·000
28-1-1937 ..	0·0381	1·0941	..	..	..	..
6-4-1937 ..	0·0381	1·0313	3·0	8·5	64·0	45·000
4-5-1 937 ..	0·0388	0·9487	4·0	32·5	175·0	48·000
26-5 -1937 ..	0·0392	0·8906	4·0	66·0	245·0	46·000
14-6-1937 ..	0·0400	0·8205	3·0	85·0	295·0	42·000
22-9-1937 ..	0·0411	0·5316	5·0	94·0	330·0	40·000
27-10-1937 ..	0·0411	0·4424	5·0	68·5	290·0	35·000

Nitrogen fixed per gram of carbon oxidized=4.6 mgm. (*i.e.*, 67 lbs. nitrogen fixed per acre in the dark.).

Sterile butter set :—

The sterile butter sets have been resterilised 5 times during the course of 2 years (the exposure lasted from 26th April, 1939 to 28th May, 1941). At the end of the exposure these sets have been tested by the plating method for any bacterial contamination and have been found to be completely sterile. Water has been added whenever the mixture of oxide and butter has become dry and at that time the set has also been resterilised.

2.2 grams fresh butter+1 gm. TiO_2 +0.1 gm. ZnO+30 c.c. water.

Energy materials.	Weight of residue gm.	Total carbon unoxidised gm.	Total carbon oxidised gm.	Total nitrogen mgm.	Gain nitrogen mgm.	Efficiency.
1	2	3	4	5	6	7
		Exposed.				
Butter+Oxide						
Total carbon=0·8730	2·5083	0·5747	0·2983	2·40	2·40	8·05
Do. +2 c.c.						
Fe Cl_3 (=0·0501 gram Fe_2O_3)	2·4948	0·5633	0·3097	2·63	2·63	8·50
		Dark				
Butter+Oxide						
Total carbon=0·8730	3·0182	0·7344	0·1386	0·65	0·65	4·69
Do. +2 c.c. Fe Cl_3	3·0025	0·7198	0·1532	0·80	0·80	5·22

It is interesting to note from the above experiments that even in the absence of bacteria and soil, nitrogen fixation takes place when butter is slowly oxidized on a chemical surface and the efficiency of the nitrogen fixation in sterile condition is slightly less than in soil. The nitrogen fixed on a sterile surface is also much greater in light than in the dark

Our experimental data show clearly that the available nitrogen (sum of ammoniacal and nitric nitrogen) is always greater throughout the whole time in the process of nitrogen fixation with molasses, carbohydrates and glycerol. On the other hand, with cellulosic materials and fats, the available nitrogen is less when the nitrogen fixation is active. It seems that a part of the available nitrogen is formed into proteins either as microbial tissue or exist as amino acids and proteins in the soil. Later on the protein nitrogen is made available to the plants by the oxidation of microbial tissues, amino acids etc.

The experimental results show clearly that both butter and ghee are slowly oxidized and cause nitrogen fixation, which is practically of the same order as that obtained with carbohydrates. Moreover, the nitrogen fixation in sunlight is nearly double that in the dark although the numbers of Azotobacter, total bacteria and fungi are much less in light than in the dark. The moisture content of the soil shows that it is fairly dry. Fats are believed to decompose only slowly in moist soils, and almost not at all in dry soils. According to Rubner (Arch. Hyg. 1922, *91*, 290) only 22·9 per cent of butter fat added to soil was decomposed during a period of one year and 32·8 per cent in 12

years. In our soils fats are much more readily oxidized as revealed in the above experiments. Our results show that fats are slowly oxidized even under sterile conditions causing nitrogen fixation.

Origin of Soil Nitrogen

It has already been stated that when cow dung is added to soil, it fixes the atmospheric nitrogen and that the value of cowdung lies not only in its nitrogen content but also in its power to fix atmospheric nitrogen. Field trials have confirmed this observation. That nitrogen fixation or accumulation takes place on the addition of farm-yard manure even in the soil of temperate climate is evident from the following results obtained in the classical field trials at Rothamsted.

	Total Nitrogen in 1914
1. Receiving no manure since 1843	.. 0·095 per cent.
2. Receiving farm-yard manure since 1852	.. 0·256 per cent.
3. Receiving complete artificials including $(NH_4)_2SO_4$	.. 0·099 per cent.

Moreover, we have shown that molasses, different carbohydrates, glycerol, dextrin, cellulosic materials like paper, hay, leaves, fats like butter, *ghee* (clarified butter), salts of palmitic, oleic, stearic, citric, tartaric, malic, oxalic, acetic acids, etc., when mixed with soil or chemical surfaces like ZnO, Al_2O_3, MnO_2, Ni_2O_3, CoO, CuO, etc. can fix atmospheric nitrogen both under sterile and unsterile conditions in presence of air. It has also been observed that the amount of nitrogen fixed per gram of carbon oxidized is much greater in light than in the dark, because light is actually utilized in nitrogen fixation.

Russell[12] has reported that the nitrogen content of a grass land increased from 0·152 per cent in 1856 to 0·338 per cent in 1912. Similarly a land permanently covered with vegetation for 24 years showed an increase from 0·108 per cent to 0·145% of total nitrogen.

The foregoing observations clearly show that carbonaceous substances help in the accumulation of nitrogen and its fixation and this explains why organic substances are valuable in steadying crop yield as observed in Rothamsted and other places.

Our results show that the loss of nitrogen chiefly due to nitrification when ammonium sulphate is added to the soil is appreciably minimised by the addition of carbonaceous substances, which act as negative catalysts in the oxidation reactions involved in the process of nitrification leading to a loss of nitrogen mainly in the gaseous state.

In most schemes of husbandry arrangements are made to keep up or even increase the supply of organic matter, whilst in forests the removal of leaves and other decomposable carbonaceous compounds is undesirable. Nemec studying the forests of Czechoslovakia observed that the removal of the litter lowered the nitrogen content of the soil by 58·6 per cent. Shutt's analysis of the Indian Head prairie soil, Saskatchewan shows that after 22 years' cultivation the percentage of total nitrogen dropped from 0·371 to 0·254. Here there was very little loss due to drainage; yet, only 1/3 of the lost nitrogen is recovered in the crop. Similar results have been reported with Minnesota

and Kansas soils. Great tracts in the United States and in Canada, stable in their natural state have been rendered liable to erosion by over cultivation and fallowing and by grazing. Both on the cultivated and on the grazed land, the organic matter (carbonaceous substances) of the soil was rapidly destroyed by oxidation causing a rapid loss of nitrogen as stated above. The effect has been an unprecendented destruction of the soil in the last few years not only by erosion but also by the loss of organic matter and total nitrogen due to rapid oxidation involved in the nitrification of proteins and other nitrogenous compounds.

In our recent experiments on nitrogen fixation the following results have been obtained:—

With numerous carbohydrates it has been found that even under completely sterile conditions the nitrogen fixed per gram of carbon oxidized in light in quartz vessels with soil is 12.2 milligrams, in glass vessels in light it is 11 milligrams, whilst in the dark it is approximately 4·8 milligrams per gram of carbon oxidized. The order of these fixations under sterile conditions is practically the same as obtained in unsterile soils. With oxides like ZnO, Al_2O_3 , Fe_2O_3, Ni_2O_3, CoO, MnO_2, etc. used as chemical surfaces instead of soil and glucose as energy material the nitrogen fixation per gram of carbon oxidized is of the order of 15 to 18 milligrams in light under completely sterile conditions, whilst in the dark it is 8 to 11 milligrams. Under unsterile conditions the fixation in light varies from 30 to 46 milligrams and in the dark from 15 to 24 milligrams per gram of carbon oxidized. The high values under unsterile conditions are being confirmed by further experiments Moreover, recent experiments carried on with sterile and unsterile soils mixed with carbohydrates and other energy materials show that the velocity of the oxidation of carbon in the carbonaceous compounds under completely sterile conditions is about 1/3rd to 1/2 of that under unsterile condition. It seems that nitrogen fixation can take place not only with the help of Azotobacter but also in the complete absence of bacteria as a surface process aided by sun-light and the chief source of nitrogen in soils in all countries is this type of fixation and not legumes as is believed to be in temperate climates. This conclusion is partly corroborated by the following observation of Morse with soils containing approximately 0.15 per cent total nitrogen. (Compare Jacks, "report of the progress of applied chemistry" 1939, vol. XXIV page 477).

Analysis of soil from experimental plots of 12 years' standing in Massachusetts showed no evidence of accumulation of nitrogen by the application of nitrogen fertilisers or by the growth of leguminous crops, nor did continuous production of nonlegumes appear measurably to deplete the soil of nitrogen. Soil nitrogen varied irregularly from year to year, showing no special tendency to increase or decrease in any plot.

The higher value of the efficiency of nitrogen fixation obtained with oxide surfaces as compared with that obtained in soils is due to the fact that the phenomena of nitrogen fixation and nitrogen loss go on simultaneously the fixation process is being opposed

by the loss due to nitrification. The unstable substance, ammonium nitrite, is formed in the process of oxidation involved in the nitrification of the proteins and other nitrogenous compounds produced by fixation or originally present in the system. But in soils, there is already a certain amount of combined nitrogen, *i.e.*, to the extent of 0.04 to 0.05 per cent in tropical soils and hence the loss of nitrogen is more marked than when oxide surfaces are used containing no nitrogen than in the soils containing nitrogen. These observations are of fundamental importance in explaining the evolution of fertile soils from the parent rocks of geological ages. The geological deposits, *i.e.*, the parent materials of soil do not contain any organic matter, but can contain inorganic nitrogen in the form of nitrates or of ammonium salt in very small quantities. The nitrates can under the influence of light and moisture and seeds form the first set of vegetable or plant life or alga, the nitrogen need of which is met from the inorganic nitrogen originally present in rocks in small quantities. The carbohydrates and cellulosic materials or other energy materials formed in photosynthesis undergo decomposition in course of time and are oxidized causing nitrogen fixation in this process which is aided markedly by light absorption and thus the store of nitrogen in the system is increased. This in its turn leads to a more abundant growth of vegetation and this process goes on in which the carbon and nitrogen status of the system is improved leading to the formation of a fertile soil. This nitrogen fixation which in the beginning at any rate is a non-biological surface reaction is aided by light absorption, because light is utilized in this process. As the original mineral is poor in nitrogenous compound the nitrogen fixation is greatly enhanced in the beginning but with the storing up of nitrogen the efficiency falls off and thus the nitrogen and carbon status of the soil reaches a maximum limit depending on the climate of the region. As a matter of fact much more nitrogen is fixed in soils in this manner by light absorption than the amounts fixed in the industrial processes taken together.

The organic matter in the soil may also be caused by the growth of alga. It is believed to be valuable in most circumstances but on newly formed soil it is of the greatest importance. The carbon of the algae when decaying is oxidized and the energy liberated leads to the fixation of atmospheric nitrogen.

The residual effect of cow or farmyard manure as observed throughout the whole world may be due not mainly to the conservation of nitrogen as hitherto believed but is caused by the fixation of atmospheric nitrogen through the oxidation of energy materials like pentosans, celluloses, fats, etc., and aided by the absorption of sun light. It seems that wherever a residual effect of a manure has been observed *e.g.* with molasses, hay or cow or farm yard manure, it is chiefly due to nitrogen fixation in the soil and perhaps no residual effect will be observed with a manure which is incapable of fixing atmospheric nitrogen on the soil surface although it may contain carbon. It seems that so far no worker has reported that legumes leave as much residual effect as cow or farmyard manure or molasses.

Improvement of Nitrogen Status of Soil

In Indian soils under cultivation as well as in those of other tropical countries the total nitrogen is much lower than in the soils of temperate countries. This is due to the fact that the prevailing high temperature and intensity of sunlight accelerates the oxidation of carbonaceous and nitrogenous compounds in tropical soils, thus entailing a loss of nitrogen caused by the nitrification of protein and other nitrogen compounds. But when the land is covered with grass or other vegetation this oxidation can be retarded and the loss of nitrogen diminished with the result that the nitrogen status can be raised. With cultivation, however the nitrogen level again falls down to the normal. By the addition of organic matter or covering with grass the nitrogen status is raised both due to the fixation of atmospheric nitrogen and to its conservation in soil.

There is, however, one advantage of the high intensity of light and heat falling on tropical soils. The available nitrogen, *i.e.*, the sum of ammoniacal and nitric nitrogen is much greater in tropical soils than in temperate ones, because of the greater facility of the oxidation of organic and nitrogenous substances in the former. Thus while the available nitrogen in tropical soils as observed by us is about 10 to 30 per cent of the total nitrogen (0.04-0.05 per cent.) the amount of available nitrogen in temperate country soils is only 1 to 2 per cent. of the total nitrogen (0.1-0.2 per cent.) present in the soil. This perhaps explains why a better crop can be grown in unmanured fields in tropical countries than in the unmanured fields of temperate countries under comparable conditions. Assuming that an acre of normal soil weighs upto nine inches depth 1000 tons, the amount of total nitrogen in European soils will be about 2.250 pounds per acre while in Indian soils it is approximately half, *i.e.*, 1.125 lbs. per acre. But the amount of available nitrogen per acre in European soils may be only 22.5 lbs. whilst in Indian soils it is about 112.5 lbs. The concentration of the dissolved nitrate and ammonium salts in the soil solutions in tropics is greater than in temperate climates and hence the absorption of these nitrogenous compounds by plant roots is much greater in a short time than in temperate climates. By adding molasses or easily oxidizable carbonaceous substances like cow-dung, hay, etc., to the soil the nitrogen status is improved by nitrogen fixation. Along with the improvement in the nitrogen status of the soil the available nitrogen also increases, thus making the soil more fertile. From our experiments with numerous molassed fields in Allahabad and elsewhere we have observed that after a month or five weeks of the addition of molasses not only is the total nitrogen content of the soil the highest but also the nitric and ammoniacal nitrogen content of the soil is the largest; and that is the time when crop has to be sown in the field.

In temperate climates an attempt should be made to improve the available nitrogen status by breaking the soil and exposing it to light and air in the spring, summer or autumn when the sun-light is of high intensity. This will, no doubt, lead to an increase in the amount of ammonium salts and nitrate but during the process of nitrification a

certain amount of nitrogen may be lost in the gaseous state. But this loss may not affect appreciably the soil fertility because of the large amount of total nitrogen present in such soils. Loss due to the escape of free ammonia cannot be marked specially in temperate climate in view of the fact that such soils have a tendency to be acidic. Instead of adding ammonium sulphate to soils in temperate countries to increase the available nitrogen it may perhaps be less expensive to increase the ploughing and breaking up of the soil, making conditions more favourable for oxidation and nitrification and obtaining a better crop yield without making the soils more acidic, as happens on the addition of ammonium sulphate. In the case of soils in temperate climates which have deteriorated and may have been given up for the purpose of cultivation it seems certain that the remedy lies in the addition of cow-manure (farm-yard manure) or other readily decomposable carbonaceous substances like molasses, hay, etc., but perhaps not by the addition of legumes, which seem to have not much residual effect on soils. If quick result is not expected, wasted or deteriorated land can be improved from the nitrogen view point by covering it with vegetation or grass and kept undisturbed for a number of years. In this way the carbonaceous substances from the decaying vegetation are oxidized and help in the fixation and conservation of soil nitrogen and thus the nitrogen status is improved.

In order to save nitrogenous manures from marked loss due to the escape of gaseous nitrogen, organic matter (carbohydrates, fats, celluloses, etc.) must be present or added to the soil especially in arid regions and under tropical conditions. Carbohydrates, celluloses and fats have been found by us to prevent the loss of nitrogen from organic nitrogenous matter and ammonium sulphate and other ammonium salts. The value of organic matter, therefore, is to help the fixation of nitrogen and protection of the soil nitrogen by stopping the loss of nitrogen gas due to the formation and decomposition of the unstable substance, ammonium nitrite. Both carbohydrates and fats are known to preserve proteins in the animal body. Similarly these substances as well as the celluloses have been found by us to preserve soil nitrogen and hence in arid conditions organic matter should always be added to the soil to prevent the loss of nitrogen and thus a mixture of ammonium salts and organic matter has been generally found to be a better manure than ammonium salts alone. This point has been well brought out by experiments all over the world. Organic matter surely leads to nitrogen fixation from the air and nitrogen protection and that is why it is such a valuable commodity for the soil.

Our experiments on nitrogen fixation have been corroborated by Dr. H.W. Kerr at Brisbane, by Dr. Shri Ranjan and Mr. Bhattacharya of the Botany Department, Allahabad University, by Mr. B. Ramamoorthy of the Imperial Institute of Agricultural Research, New Delhi, and by Mr. Sulaiman of the Dacca University, Dacca. It is stated that results obtained in Hawaii (Handbook of *Hawaiian* soils p. 190 ; also International sugar Journal, 1937, 39, 419, 420) inexplicable under the biological hypothesis can be explained by the photochemical hypothesis of Dhar and co-workers.

References

1. Iodin Compt. rend. *55*, 612 (1862).
2. M. Berthelot Compt. rend. *101*, 775, (1885).
3. H. Hellreigel and H. Wilforth Z. Vereins, Ruberzucker Industries, (1888).
4. S. Winogradsky, Compt. rend. *116*, 1385, (1895).
5. M. V. Beijerinck (Zbl. Bakt. 7, 561, 1901).
6. N. R. Dhar Presidential address to the National Academy of sciences, India, December 1935 and January 1937 ; Proceedings National Academy of sciences, India *11*, 97, 1941 ; Nature *134*, 572 (1934) ; 138, 1060 (1934).
7. N. R. Dhar and S. K. Mukherjee J. Indian Chem. Soc. *11*, 727 (1934).
8. Iodin J. Amer. Chem. Soc. *32*, 396 (1910).
9. Schreiner and Skinner U. S. Dept. Agric. Bur. Soils Bulletin 87 (1912).
10. Lathrop. J. Franklin, Inst., 169, 183, 303, 465 (1917).
11. S. A. Waskman, Principles of soil microthology, (1927).
12. E. J. Russell, Soil conditions and plant growth 362 (1934).